The Journal of Laboratory and Clinical Medicine

VOL. 22 AUGUST, 1937 NO. 11

CLINICAL AND EXPERIMENTAL

EXPERIMENTAL STREPTOCOCCUS INFECTIONS IN RABBITS FOR THERAPEUTIC INVESTIGATIONS*

JOHN A. KOLMER, M.D., AND ANNA M. RULE, PHILADELPHIA, PA.

THE work described in this paper is the result of an effort to produce experimental streptococcus infections in rabbits suitable for therapeutic investigations. The problem has been the production of a primary local lesion permitting direct observation as well as the production of a focal lesion associated with bacteriemia and metastatic infections in order to approximate the usual severe streptococcus septicemia of human beings. In other words, the production of bacteriemia alone does not appear to be sufficient, as the problem of chemotherapy is not only disinfection of the blood but much more importantly, disinfection of the primary and secondary infections of the fixed tissues.

Mice inoculated intraperitoneally with highly virulent strains of streptococcus have been usually employed, but in our experience either a too rapidly fatal septicemia or no septicemia at all has usually resulted. Morgenroth[1] has employed mice inoculated subcutaneously for the production of a focal lesion and septicemia which has proved a better procedure than intraperitoneal infections[2] but in our experience a sufficiently virulent strain produces fatal infection too rapidly for therapeutic investigations since the severity of the infection does not usually permit the administration of repeated doses of the agent under study and may mask any slight curative activity insufficient to produce complete disinfection in one or two doses at daily intervals.

Since we have found Goodner's[3] intradermal infection of rabbits with pneumococcus[4] a very helpful experimental lesion for serum and chemothera-

*From the Research Institute of Cutaneous Medicine.
Received for publication, December 16, 1936.

peutic investigations, we have inquired into the possibilities of using a similar method with streptococcus. With sufficiently virulent strains of hemolytic types, severe focal lesions have been produced but rarely associated with bacteriemia and, consequently, not fatal in outcome. As in the case of the pneumococcus infection, the local lesions however, permit direct observations on the influence of any therapeutic agent being administered. By combining intradermal with intravenous injections of virulent streptococci, a severe local and systemic infection may be produced usually resulting in bacteriemia and suppurative arthritis and also constituting a satisfactory experimental infection for therapeutic investigations.

EXPERIMENTAL METHODS

Streptococcus Cultures.—The hemolytic streptococcus (Group A) used in these experiments was originally isolated from a throat three years ago. Since then additional strains of sufficient virulence have been obtained from various human sources, and recently from a case of puerperal sepsis. Only strains sufficiently virulent for mice to produce rapidly fatal septicemia following the intraperitoneal injection of 0.001 c.c. or less of eighteen-hour hormone broth cultures have been found satisfactory. Virulence has been maintained by frequent mouse or rabbit transfers. It is apparent, however, that the natural resistance of the adult rabbit to intradermal inoculation is much higher than that of the mouse to intraperitoneal infection. For example, while 0.001 c.c. of eighteen-hour hormone broth culture may produce rapidly fatal septicemia in the latter corresponding to about 0.05 c.c. per kilogram, well-pronounced intradermal lesions required the injection of 0.6 c.c. in rabbits weighing from 1,800 to 2,400 gm. Furthermore, the resistance of adult rabbits to intradermal infection alone, as well as to combined intradermal and intravenous infection, has been found to vary, as some animals developed more severe infections than others when infected with approximately similar amounts of culture per body weight, but the variations in therapeutic experiments have been readily controlled by including a sufficient number of untreated control animals.

Rabbits.—Full-grown healthy rabbits (1,800 to 2,400 gm.) have been found more satisfactory than younger animals. White or light gray animals are preferred. The hair of the abdominal skin was removed with barium sulphide. After preliminary temperature and leucocyte observations the streptococci were injected.

Production of Intradermal Infection.—The site of infection was the midline about midway between the thorax and pelvis; 0.6 c.c. of eighteen-hour hormone broth culture is injected intradermally being divided into three injections of 0.2 c.c. closely spaced since the skin is too thin to accommodate more than this amount of inoculum. As shown microscopically however, it is impossible to inject 0.2 c.c. strictly intradermally because the layer of epidermis is too thin to accommodate this amount of inoculum. As a result, the larger portion is deposited beneath the epithelial layer.

Production of Intradermal and Systemic Infection.—The intradermal infection is given in the same manner as above, followed four hours later by a single intravenous injection of 0.5 c.c. of the same culture.

Observations.—Beginning two to four hours later, frequent observations of the local intradermal lesion are made, particularly as to its size, color, amount and consistency of swelling along with morning and evening temperatures and daily leucocyte counts. In the combined intradermal and systemic infections, the joints are examined for signs of suppurative arthritis among animals surviving seven to ten days or longer when these lesions are usually detectable by clinical examination.

Blood and Lesion Cultures.—Using a small syringe and needle about 0.2 to 0.3 c.c. of blood was removed aseptically from a marginal ear vein and planted in a tube of hormone broth. No attempt was made to plate blood for numerical counts. These cultures were made daily or at frequent intervals.

The local lesion cultures were made daily by aspirating fluid aseptically with a small needle and inoculating tubes of hormone broth. Since the lesions usually contained but little fluid (usually much less than observed in pneumococcus lesions) these were not always successful and particularly after the fourth or fifth day. Joints were aspirated and cultured in the same manner, using needles sufficiently large for the aspiration of pus.

THE LOCAL LESION PRODUCED BY INTRADERMAL INFECTION

The lesion usually makes its first appearance about 2 to 4 hours later as an inflamed swollen area from 1 to 2 cm. in diameter. It progresses rapidly, reaching its maximum size in twenty-four hours and measuring about 5 by 6 cm. At this time it is markedly swollen by the accumulation of cloudy serous exudate and leucocytes and of red, angry appearance sometimes hemorrhagic and especially in the central portions, fading to pinkish color at the periphery (Fig. 1). The administration of certain new chemical agents sometimes produced a temporary exacerbation of these changes.

The lesion usually reached its maximum size in twenty-four to thirty-six hours after infection, when regression usually begins. The lesion does not leak serum, shows no tendency to break down and but rarely becomes contaminated with staphylococci or other organisms from the skin.

In from four to six days most of the swelling has disappeared but a local abscess with pus and superficial crusting frequently developed at this time. Healing usually occurred in from ten to fourteen days after inoculation.

For purposes of record the severity of the local lesion was conveniently recorded as ++++, to express the maximum of severity, with +++, ++, and + expressing progressing less severe lesions, and – designating practically complete healing.

In no instance was this local infection found to produce arthritis or metastatic infection in the other organs and tissues.

None of our animals succumbed from this local infection alone.

In no instance were the lymphatic glands of the groin or axillae demonstrably enlarged or suppurative.

Microscopic Examination of the Local Lesion.—Histologic examination of tissues removed twenty-four hours after inoculation, when the lesions were at

about the maximum of intensity, showed that the epidermis is not involved but that the corium is enormously infiltrated with polymorphonuclear leucocytes with small amounts of serofibrinous material. There is some congestion of blood vessels and lymphatics and the subcutaneous connective tissues are swollen and slightly edematous. In some instances the leucocytic infiltration and edema involve the superficial muscle layers. Sections have usually shown an abundance of streptococci in the exudates, some of which are intracellular.

Lesion Cultures.—Cultures of fluids aspirated from the local lesions twenty-hours after infection were invariably positive and daily cultures usually continued positive for 3 to 6 days and sometimes even when the lesions were

Fig. 1.—A photograph of a focal lesion twenty-four hours after intradermal infection.

healed to +. Technical difficulties and healing sometimes prevented successful aspirations after the third or fourth day, but in general terms it appeared that streptococci tended to persist in the lesions until healing was far advanced and almost complete.

Bacteriemia.—Blood cultures were only occasionally and irregularly positive in the course of the intradermal infection. In the majority of animals they were negative throughout. Occasionally positive cultures were found in animals receiving intravenous injections of new chemical compounds and especially in the case of those producing temporary exacerbation of the local lesions.

Temperature Reactions.—All of our temperatures were sublingual, as we found these more regular and satisfactory than rectal temperatures. The normal temperature by this route was found to vary from 101° to about 102° F.

About twelve hours after infection the temperature rises to about 103° or 104° F., reaching the maximum (103° to 105° F.) at the end of twenty-four to thirty-six hours followed by rapid regression to pre-infection levels at the end of forty-eight to seventy-two hours as shown in Chart 1 representative of the usual temperature curve.

Occasionally in severe infections this drop in temperature has been followed by secondary elevations, each one becoming progressively less as shown in Chart 2, although not always accompanied by demonstrable exacerbation of the local lesion.

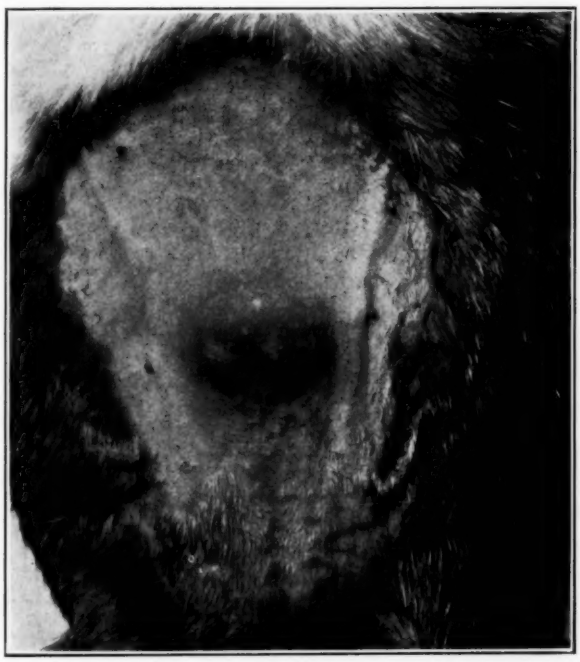


Fig. 2.—A photograph of a focal lesion forty-eight hours after intradermal infection.

Leucocyte Counts.—As is well known the leucocyte counts on rabbits are generally regarded as subject to great fluctuation. The average count on our normal fasting animals has varied from about 7,000 to 12,000 per c.mm., but repeated counts on individual rabbits undergoing handling has shown that it is difficult to establish a relative normal for any individual animal.

Daily counts before and after inoculation however, have shown leucocytosis usually reaching a maximum in from twenty-four to forty-eight hours after infection and following fairly closely the temperature elevations. As shown in Chart 1, the leucocyte counts generally reach from 14,000 to 16,000 per c.mm. at this time, falling to normal levels by the third or fourth day but subject to secondary increase in the case of those animals having secondary elevations of temperature (Chart 2).

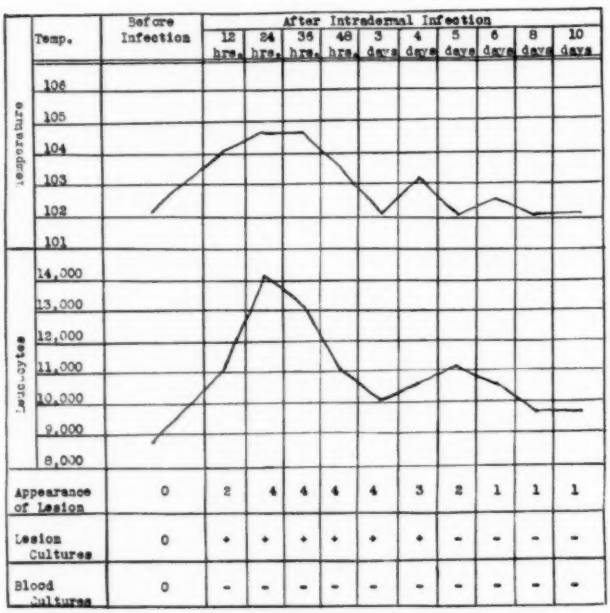


Chart 1.

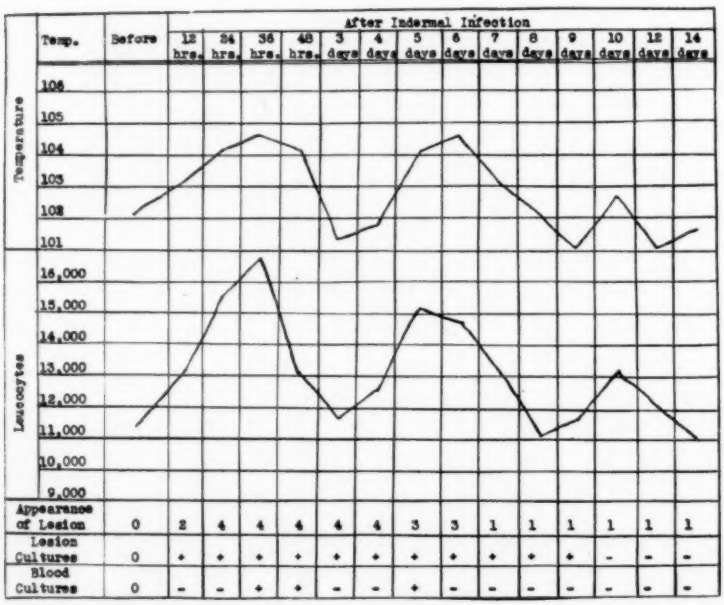
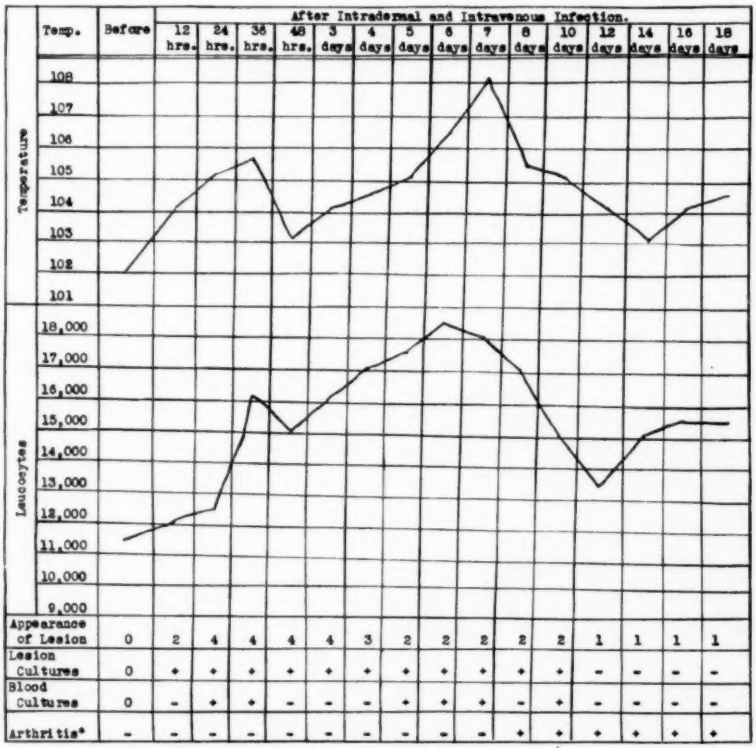


Chart 2.

THE COMBINED INTRADERMAL AND SYSTEMIC INFECTION

As previously stated this was produced by the intradermal injection of 0.6 c.c. of eighteen-hour hormone broth cultures in divided amounts followed four hours later by the intravenous injection of 0.5 c.c. of the same culture.

The local lesions and cultures were closely similar to those described. In other words, the supplementing intravenous inoculation did not appear to render the local lesions any less severe but positive blood cultures were much more frequent and especially during the first three to four days following infection.



Temp.	Before	After Intradermal and Intravenous Infection. 12 hrs.	24 hrs.	36 hrs.	48 hrs.	3 days	4 days	5 days	6 days	7 days	8 days	10 days	12 days	14 days	16 days	18 days
Appearance of Lesion	0	2	4	4	4	4	3	2	2	2	2	2	1	1	1	1
Lesion Cultures	0	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-
Blood Cultures	0	-	+	+	-	-	-	+	+	+	-	+	-	-	-	-
Arthritis*	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+

* Left hind knee, ankle and hip joints (cultures positive).

Chart 3.

The temperature and leucocytic changes however, were more irregular and usually much more pronounced as shown in Chart 3 representative of the series.

This combined infection however, was usually much more severe than the intradermal infection alone since many of the animals succumbed within twenty-one days and sometimes as early as forty-eight hours after infection.

The most characteristic features were the almost invariable development of bacteriemia in twenty-four hours, persisting for three to six days or longer, followed by the development of suppurative arthritis among the majority of surviving animals in from five to ten days after infection and persisting for

as long as one to five months. When arthritis was well developed the blood cultures were usually sterile thereafter.

The joints of the front and hind legs including the hips were those usually involved, with no regularity in the distribution of the lesions.

Among surviving animals the arthritis became suppurative with invariably positive joint cultures, but in no instance have we observed spontaneous discharge of pus.

Upon death, autopsy has frequently shown the presence of serofibrinous fluid in the pleural and peritoneal cavities, with positive cultures. No other gross lesions were found.

OBSERVATIONS ON SERUM THERAPY

Our main interests have been in chemotherapeutic studies with new synthetic chemical agents in the treatment of these experimental streptococcus infections,[5] but we have also tried some of the commercial antistreptococcus sera in the treatment of the focal lesion produced by intradermal infection.

The results of one of these experiments with two polyvalent concentrated antistreptococcus antitoxins and two whole sera are summarized in Table I. Erysipelas antitoxin was not employed.

TABLE I

EFFECT OF POLYVALENT ANTISTREPTOCOCCUS SERUMS UPON THE INTRADERMAL INFECTION

SERUMS	DOSE PER KILO (C.C.)	NUMBER DOSES*	LESIONS; DAYS							LESION CULTURES; DAYS						
			1	2	3	4	5	8	14	1	2	3	4	5	8	14
(A) Concentrated	1.0	5	4	4	3	2	2	2	1	+	+	+	+	+	+	-
(A) Concentrated	1.0	5	4	4	4	3	2	2	1	+	+	+	+	-	-	-
(A) Concentrated	0.5	5	4	4	3	3	2	2	1	+	+	+	+	-	-	-
(B) Concentrated	1.0	5	4	4	4	3	0			+	+	+	+	0		
(B) Concentrated	1.0	5	4	4	3	2	2	1	1	+	+	+	-	-	-	-
(B) Concentrated	0.5	5	4	4	4	3	2	1	1	+	+	+	+	-	-	-
(C) Whole	1.0	5	4	2	1	-	-	-	-	+	-	-	-	-	-	-
(C) Whole	1.0	5	4	2	1	-	-	-	-	+	-	-	-	-	-	-
(D) Whole	5.0	5	4	4	3	2	2	2	1	+	+	+	+	+	-	-
(D) Whole	2.0	5	4	4	4	3	1	1	1	+	+	+	-	-	-	-
(D) Whole	1.0	5	4	4	4	3	2	1	1	+	+	+	+	-	-	-
Control	-	-	4	4	4	4	3	2	1	+	+	+	+	+	-	-
Control	-	-	4	4	4	2	2	1	1	+	+	+	+	-	-	-
Control	-	-	4	4	4	4	4	3	3	+	+	+	+	+	+	-

*By intravenous injection. First dose given four hours after intradermal infection, followed by four additional doses at daily intervals.

Each serum was given by intravenous injection, the first dose being administered four hours after intradermal infection when the local lesions were already developing, followed by four additional doses at daily intervals. The amounts administered varied from 0.5 to 5.0 c.c. per kilogram of weight, as shown in the table.

No appreciable effects were observed on the clinical course of the local lesions or upon the lesion cultures by three of these serums (A, B and D) but serum C appeared to exert a definite therapeutic influence since the local lesion rapidly regressed along with apparent rapid destruction of streptococci in the lesions (negative cultures). Under the circumstances, it may be in-

ferred that this particular serum contained therapeutic amounts of antibody for the particular strain of streptococcus employed.

So far no experiments have been conducted in the treatment of rabbits infected by the combined method of intradermal and intravenous infection. It remains to be determined, therefore, whether or not antistreptococcus sera have any appreciable effect upon the bacteriemia or in the prevention of metastatic infection of the joints, but of the four employed in this work, only one hastened recovery from the intradermal lesions and exerted an appreciable influence upon the streptococci contained in them.

DISCUSSION

The chief value of these experimental lesions is that the intradermal one offers, in a suitable laboratory animal, a local lesion easily produced and observed and in many respects resembling streptococcus cellulitis of human beings, while the combined intradermal and intravenous inoculation provides a severer infection with bacteriemia and metastatic infection of the joints. Both appear to offer satisfactory lesions for the experimental study of biologic and chemotherapeutic compounds. Not only may one make fairly accurate clinical observations of the local lesion along with associated temperature and leucocytic changes, but more importantly, the possible effect of therapeutic agents upon the streptococcus contained in the local lesions as well as those in the blood and joint lesions.

SUMMARY

1. The intradermal inoculation of adult rabbits with virulent hemolytic strains of Group A streptococcus gives rise to a local lesion and a sequence of events having an analogy to streptococcus cellulitis of human beings.

2. Combined intradermal and intravenous inoculation provides a severer infection with bacteriemia and metastatic infection of the joints.

3. These symptom-complexes have been described in detail, particularly as to the development of the local lesion, the temperature reactions, the leucocytic changes, the persistence of streptococci in the lesions, and the bacteriemia.

4. Rabbits invariably recover from the local infection alone, but the majority of untreated animals finally succumb to the combined intradermal and intravenous infection.

5. These experimental lesions appear satisfactory for therapeutic investigations.

REFERENCES

1. Kolmer, J. A., Raiziss, G. W., and Rule, A. M.: The Chemotherapy of Streptococcus Infections of Mice With Special Reference to Salicyl Compounds, J. Pharmacol. Exper. Therap. **43**: 71, 1931.
2. Kolmer, J. A.: Chemotherapy With Special Reference to the Treatment of Syphilis, Philadelphia, W. B. Saunders Company, p. 79.
3. Goodner, K.: Experimental Intradermal Pneumococcus Infection in Rabbits, J. Exper. Med. **48**: 1, 1928.
4. Kolmer, J. A., and Rule, A. M.: Goodner's Intradermal Pneumococcus Infection of Rabbits, J. Infect. Dis. **57**: 47, 1935.
5. Kolmer, J. A., Brown, H., and Raiziss, G. W.: The Chemotherapy of Experimental Streptococcus Infections of Rabbits with Special Reference to Pyridine Compounds and Sulfanilamide. J. Pharmacol. & Exper. Therap. (To be published.)

THERMOREGULATION AMONG THE VISCERA WITH DESCRIPTION OF A MEANS OF PRODUCING HYPOTHERMIA IN UNANESTHETIZED ANIMALS*

JAMES B. HAMILTON, NEW HAVEN, CONN.

BY PREVIOUSLY implanted thermocouples temperature determinations can be made of internal organs in intact and unanesthetized animals.[1] These reveal a thermal uniformity of abdominal organs.[1, 2] The uterus of the rat remains in agreement with the body level of heat throughout the physiologic changes undergone in estrual rhythms, pregnancy, parturition, lactation, and involution. Similarly, the more rapid changes induced by injection of theelin and adrenalin do not affect the common organ-body level of heat.

These data represent the capacity of the uterus to respond to severe and rapid changes of the body level of heat by pharmacologic and physical means. It presents an opportunity to study the parts played by direct tissue conduction and by blood convection in the maintenance of a thermal homeostasis among the abdominal organs.

Results.—Variance in the body heat level induced by drugs.

1. *Elevation by Yeast and Dinitrophenol:* Subcutaneous injection of 10 c.c. per kilo of a 15 per cent solution of Northwestern yeast produces a steady febrile level in the rat which lasts eight to sixteen hours. Fig. 1, *A* exemplifies the uniformity of uterine and body temperatures during the rise, high level, and fall of yeast fever in 5 rats.

A dosage of 25 gm. per kilo was used for the production of dinitrophenol fever in another series of 5 rats. This dosage approaches the lethal figure suggested by Tainter, Bergstrom and Cutting,[3] but the elimination of movement by previous acclimation to immobilization cages may have tended to prevent a larger degree of rise. A thermal agreement similar to that in yeast fever is seen between uterus and body through the rise, the high level, and the fall of the dinitrophenol fever. Acetanilid acted as an antipyretic to lower the body temperature in 3 animals without disturbing the organ-body relationship.

2. *Lowering of the Level in the Rat by Methylene Blue, Cocaine, and Anesthetics:* The rat occupies a distinctive position among laboratory animals in its peculiar reaction to certain drugs, such as cocaine and methylene blue, which induce febrile conditions in the cat and dog but serve to lower body temperature in the rat. Methylene blue of M/100 solution in dosages of 10 c.c. per kilo caused a fall in the body heat level of as much as 4° F., within an hour. As in the results obtained with pyretics, a thermal equilibrium between the uterus and the body was maintained despite disturbances of the heat

*From the Departments of Anatomy, Yale Medical School, and Physiology, Albany Medical College.

Received for publication, October 1, 1936.

regulatory mechanism (Fig. 1, *B*). A general decrease in temperature obtained with cocaine confirmed the findings with methylene blue.

Anesthesia also substantially lowered body temperature in a chamber maintained at 76° F. Fig. 1, *C* shows the usual organ-body constancy which remained during a decrease, maintained low level, and subsequent increase of body heat upon administration of sodium amytal in dosages suggested by Nicholas and Barron.[4] Morphine sulphate injected subcutaneously had inconstant effects on the level of body heat but no effect upon the uniformity of visceral temperatures.

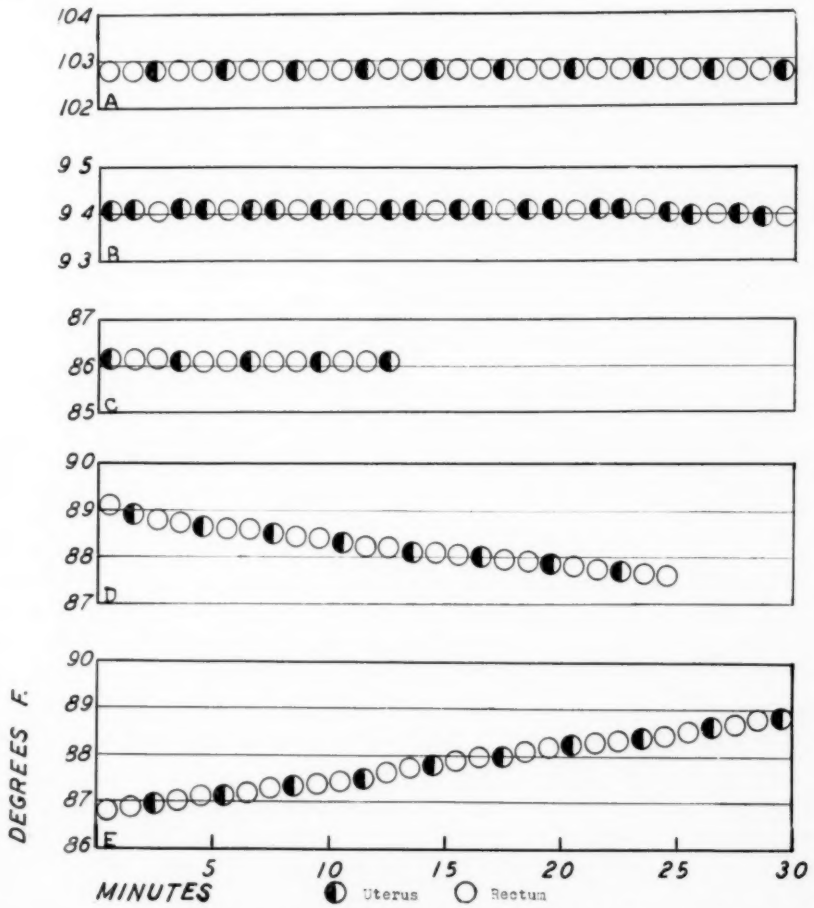


Fig. 1.—Agreement of uterine and body temperatures. *A*, In hyperthermia. Fever induced by injection of yeast. *B*, In drug-induced lowering of body level of heat (injection of methylene blue). *C*, In anesthesia (sodium amytal). *D*, In hypothermia due to cold environment. Animal unanesthetized. *E*, In the absence of blood flow. Dead animal placed in warm chamber.

3. *Variance in the Body Heat Level Induced by Extremes of Environmental Temperature:* After the physiologic changes which occur in the uterus[2] and body changes produced by drugs were found insufficient to alter the thermal equilibration of the body, more severe and marked changes in body temperature were produced.

Hyperthermia was induced either by placing the rat directly in a chamber at 95° F., or by gradually increasing the usual (76° F.) chamber warmth.

The uterine temperature agreed with the body level of heat during the hyperthermic rise, the maintained elevation and later the fall in body temperature.

Death intervenes at less than 10° F. above normal body temperature (approximately 109° F.). More conclusive data concerning uterine conformity to the body level of heat are obtained from the hypothermic states. The rodent has been found in these experiments to have an exceedingly rapid heat loss in a moderately cool environment (35° F.) without the use of anesthesia or other means of destroying body heat regulation as is necessary for rapid and pronounced heat loss in the cat, dog, and other animals. The loss of heat is abrupt and not effectively interrupted by shivering or other cold counteractant. Rats spontaneously recover from one- to two-hour exposures even though the temperature of the body decreases to the low level of 60° to 54° F., as ascertained by a deep rectal thermocouple or from readings of various internal organs. Upon removal from the cold chamber, the animal spontaneously begins the resumption of the usual level of body heat. In such hypothermic conditions the thermal relationship of the organs to the rest of the body was examined for any trace of (1) a lag in organic temperature above the general body level during heat loss, or (2) any persistently higher organic temperature at low levels of body heat, which might be taken as an indication of a distinctive source of heat production.

Care was taken in the interpretation of readings when the chamber was cold enough to produce a particularly rapid loss of body heat, for in these conditions the rectal thermocouple may register higher than the thermocouple implanted in the uterus, due to the thermal inertia of the larger mass of the rectal thermocouple which opposes any rapid change in heat level.*

The data obtained from extreme variations of the body heat level in hypothermia confirm the unbroken uniformity of uterine and body temperatures which was observed in hyperthermia and in procedures that had induced a lesser degree of change in the body level of heat. Fig. 1, *D* shows similar uterine and colonic readings in the unanesthetized rat at 66° F.

Moreover, this conformity during extreme hypothermia was never seen to vary with the amount of blood flowing through the uterus. A striking illustration of this is seen in rats subjected to hypothermia during various stages of the estrual cycle, pregnancy, and finally in the castrate condition—with an exact thermal agreement of organ and body despite great differences in uterine blood flow combined with severe changes in body temperature.

DISCUSSION

The uterus of the rat conforms to the general level of body heat during (1) the extreme physiologic changes that occur in the organ as in pregnancy and in parturition, and (2) changes in the level of body heat which affect the uterus secondarily. Further evidence will be brought forward in work now

*The testing of this theory lay in the suturing of a thermocouple from an internal abdominal approach to the intestine at a site identical with that of the rectally introduced thermocouple. Presumably, the two should register the same unless the larger mass of the rectal thermocouple causes a lag in rapid changes of body temperature. The 2 thermocouples recorded identically at any continued level of body heat and through ordinary cooling. In rapid falls, however, the rectally inserted lead lagged behind as much as a whole degree Fahrenheit.

in progress that there exists throughout the other abdominal organs an *internal integration of heat*, a homeostasis that remains undisturbed physiologically or pharmacologically. As it seems logical to assume that all internal parts do not possess the same metabolism or identical heat production, these findings of uniform abdominal warmths arouse interest as to the mode of integration of warmth from one part to another. In the presence of differential heat production, how is the integration of organ and system maintained?

In its totality, the temperature of the animal body is a resultant of the interplay between forces producing heat and forces dissipating this thermal energy from the body. In an environment lower than the body temperature, the following equation holds:

Heat Production – Heat Elimination = Body Heat.

The physical means of heat elimination from the body surface are conduction, convection, radiation and evaporation. In the elimination of heat within the body, that is, equilibration of warmth from one internal part to another, radiation and evaporation may be disregarded. The following set of experiments were devised to test conduction and convection in the internal integration of heat:

a. An organ with reduced blood flow:
   1. A hypothermic state of reduced flow through the whole body.
   2. A castrate state with reduced flow through the uterus.
   3. Castrate animals in hypothermic conditions.

b. An organ with increased blood flow:
   1. Hyperthermic states with increased blood flow through the whole body.
   2. Hormonal stimulation, estrual and pregnant conditions with increased blood flow through the uterus.
   3. Pregnant animal in hypothermic conditions.

c. Conduction by the tissues entirely apart from convection by blood flow.
   1. Lack of blood flow through the organ.
   2. Lack of blood flow through the whole body.

In a hypothermic rat with a body heat level reduced to 56° F. the heart rate is irregular, weak and only 40 per minute as compared to 200 to 400 per minute at normal heat levels. The appearance of the rat is a pale gray, bordering upon cyanosis. The eyes are whitish pink, the red color fading away with the advancing state of hypothermia. Obviously, blood flow is markedly lessened, but the organ-body uniformity of heat is not disturbed by the reduced thermal convection. The uterus of the castrate animal, despite lessened circulation through the organ, conforms to the body level of heat in a manner indistinguishable from the normal animal not only during normal conditions but also during hyperthermia, hypothermia, and rapid change in general body temperature.

Similarly, increased flow of blood through the organ (theelin injections, pregnancy) or through the body as a whole (hyperthermia)—or through both organ and body (hyperthermic animal during pregnancy)—does not affect the thermal integration of the abdominal part with the whole body. Sudden and complete elimination of uterine blood flow in the living unanesthetized

rat was not accomplished beyond doubt, but tests of at least almost entire cessation of uterine circulation further proved that conduction can to a large part thermally equilibrate organ and body in the living animal.

Since conduction from tissue to tissue and convection by blood are difficult of analysis in normal conditions, the former was tested alone in the dead animal. Even in the entire absence of blood flow the uterine and rectal temperatures are identical at any level of body heat (Fig. 1, *E*). Even during environmentally produced changes in the level of the dead animal's body heat, conduction alone suffices to keep the organ and internal body at the same temperature.

These results are relevant to (1) an understanding of organic temperatures during fever, and (2) an evaluation of the thermal changes in the viscera that may be expected from the application of hot and cold water bags to the skin, diathermy, radiothermy, and the Elliot intrapelvic devices. In view of the great capacity for thermoregulation among the viscera of rodents and of larger animals,[1] the spread of heat from local thermal applications to the skin and to sites reached through the orifices of the body is seen to be rapid and, to a considerable extent, a result of tissue conduction as well as of blood convection.

SUMMARY

1. In small animals a cool environment (35° F.) is sufficient to produce hypothermia (60° F.) rapidly even in the unanesthetized animal.

2. The temperature of the rat uterus is maintained constantly at the level of body heat (colonic) during:

a. Elevation of body temperature by yeast and dinitrophenol.

b. Lowering of body temperature by methylene blue, cocaine and anesthesia.

c. Severe hyper- and hypothermia (colonic temperatures of 109° to 55° F.) induced by environmental exposure.

3. This thermal uniformity of organ and body is independent of the amount of the uterine blood flow as seen in conditions of pregnancy, castration and absence of blood flow.

4. Indication is given of the significance of direct tissue conduction (a) in the integration of the heat produced by the abdominal organs to form an identical uterine-body level of heat and (b) in the thermal homeostasis which counteracts attempts to change the temperature of a part of the body as by hot or cold applications to the skin, diathermy, or radiothermy.

REFERENCES

1. Hamilton, J. B.: Visceral Temperatures in the Intact and Unanaesthetized Animal. I. A New Technique for Measurement, J. Lab. & Clin. Med. **22**: 466, 1937.
2. Idem: Visceral Temperatures in the Intact and Unanaesthetized Animal. II. The Uterus of the Rat, Endocrinology. In press.
3. Tainter, M., Bergstrom, F., and Cutting, W.: Metabolic Activity of Compounds Related to Dinitrophenol, J. Pharmacol. & Exper. Therap. **53**: 58, 1935.
4. Nicholas, J., and Barron, D.: The Use of Sodium Amytal in the Production of Anaesthesia in the Rat, J. Pharmacol. & Exper. Therap. **46**: 125, 1932.

THE FILTRABILITY OF BILIRUBIN IN OBSTRUCTIVE JAUNDICE*

RAYMOND L. GREGORY, PH.D., M.D., NEW ORLEANS, LA., AND
MARIE ANDERSCH, PH.D., PHILADELPHIA, PA.

AS THE result of their work concerning dialysis of plasma bilirubin, Hoover and Blankenhorn[1] concluded that there was a close analogy between the behavior of plasma bilirubin toward the glomerular filter and collodion membranes. We quote from their paper: "We find an unvarying consistency in the behavior of bilirubin toward the collodion dialyzer (with rather thick wall) and toward the renal filter.

"If a patient has bilirubin in his blood and none in his urine, the dialysate from his plasma will contain no bilirubin. If bilirubin occurs in both plasma and urine, the dialysate will contain bilirubin." They found one exception to this in a patient who had renal damage, and they ascribe the discrepancy to this cause. Blankenhorn[7] subsequently found that there may be dialyzable and nondialyzable fractions of bilirubin in both obstructive and acholuric types of jaundice. Leschke[8] confirmed the work of Hoover and Blankenhorn.[1] In his review on bilirubinemia, Barron[9] states that he has been unable to find any difference in the dialyzability of bilirubin in obstructive or hemolytic jaundice. He refers to no published work and gives no evidence for his statement.

We wish to submit experiments the results of which demonstrate that bilirubin will not dialyze or ultrafilter from serum, from urine, or from solutions of bilirubin, through collodion membranes of such pore size that they allow free ultrafiltration of inorganic ions but retain protein.

PLAN OF STUDY

Patients with a high immediate direct van den Bergh value for the serum bilirubin, bilirubinuria, and obstructive disease of the extrahepatic bile ducts proved at operation furnished serum and urine for study. Both the serum and urine were highly pigmented, and the latter gave positive Gmelin tests for bilirubin. Both serum and urine were subjected to ultrafiltration in collodion sacs at a negative pressure of 150 mm. of mercury. In some instances, they were also dialyzed in cellophane tubes against distilled water for periods up to twenty-four hours.

Gallbladder bile obtained at autopsy was filtered to remove the mucus. This was dissolved in normal serum. The resulting solution was ultrafiltered and dialyzed.

Finally, purified bilirubin was prepared, dissolved in sodium hydroxide and solutions of desired concentration in water and normal serum were made. These solutions of purified bilirubin were subjected to dialysis and ultrafiltration.

*From the Department of Internal Medicine, State University of Iowa, Iowa City, Iowa.
Received for publication, December 17, 1936.

METHODS

The ultrafiltrations were done through collodion sacs made according to Greenberg and Gunther;[2] and according to the method previously described by us.[3] The purified bilirubin was prepared and the serum bilirubin was determined by the van den Bergh method as modified by Gibson and Goodrich,[4] Thannhauser and Anderson[5] and Newman.[6] The cellophane employed was the DuPont product of tubular design and about three-fourths inch in diameter. The solution to be dialyzed was placed in a loop of this and both ends were tied up out of the fluid, against which the serum or urine was being dialyzed. In some instance (see Table I), the serum and urine and bilirubin solutions were ultrafiltered in the collodion sacs, following which they were dialyzed against distilled water in the same sac. The volume of the distilled water against which the serums and urines were dialyzed was 25 c.c. in each instance. Eight to 10 c.c. of serum or urine were employed in each ultrafiltration or dialysis.

An "artificial" jaundiced serum of high bilirubin content was prepared by dissolving gallbladder bile in pooled normal serums. This was studied in the same manner as the above.

RESULTS

Table I shows the results of our studies of the serum and urine in 5 cases of jaundice with bilirubinuria, all showing van den Bergh reactions of the direct immediate type.

No bilirubin went through the collodion membrane in any of the 5 cases, although the bilirubin in the blood ranged from 15.8 to 24 mg. per cent and there was bilirubin in the urine. Furthermore, bilirubin which had passed through the kidney would not filter through the collodion membrane, demonstrating that the filtration through the kidney and through collodion membranes is not comparable.

Serum with the bilirubin augmented by the addition of gallbladder bile until the concentration of the bilirubin was 34.8, likewise gave an ultrafiltrate containing no bilirubin.

It is also noteworthy that water solutions of purified bilirubin gave a clear filtrate although no protein was present to "bind" the bilirubin and prevent its passage through the filter.

The results on solutions of the sodium salt of bilirubin and on "artificial" jaundiced serum are tabulated in Table II.

COMMENT

We are unable to explain the lack of agreement between our results and those of Hoover and Blankenhorn and of Leschke in regard to the dialysis of bilirubin from icteric serum obtained from patients who showed hyperbilirubinemia and bilirubinuria. The pore size of their membranes may not have been carefully controlled and some of these may have had pores large enough to allow bilirubin to go through. We have found that if the membranes allowed traces of protein to pass, bilirubin would also go through.

The results of the present experiments clearly indicate that bilirubin of blood serum and urine in obstructive jaundice, bilirubin in gallbladder bile,

TABLE I

CASE	BLOOD SERUM				URINE				
	VAN DEN BERGH MG. %	ULTRA-FILTRATE	DIALYSATE		COLOR	VAN DEN BERGH	GMELIN (HNO_3)	ULTRAFILTRATE	DIALYSATE
			COLLODION	CELLOPHANE					COLLODION
S	24.0	Colorless	Colorless 12 hours	Not done	Highly pigmented	10 mg. %	+	Normal urine color. Negative Gmelin and diazo.	No color 12 hours
L	19.5	Colorless	Colorless 24 hours	Colorless 24 hours	None obtained				
P	34.0	Colorless	No color 8 hours; slight yellow tinge 24 hours. Negative HNO_3 and diazo.	Not done	Very dark		+	Normal urine color. Negative HNO_3 and diazo.	No color 12 hours
H	15.8	Colorless		Not done	Highly pigmented	Qualitative positive	+	Normal urine color. Negative HNO_3 and diazo.	
R	17.4	Colorless		Not done	Highly pigmented		+	Normal urine color. Negative HNO_3 and diazo.	

TABLE II

	VAN DEN BERGH MG. %	ULTRAFILTRATE	DIALYSATE	
			COLLODION	CELLOPHANE
"Artificial serum"	34.8	Colorless	Colorless 20 hours	Colorless 20 hours
Bilirubin Solution A	2.0	Colorless		
Bilirubin Solution B	8.8	Colorless		
Equal parts of normal serum and Bilirubin B	Approx. 5.0	Colorless		

and purified bilirubin in aqueous and serum solutions do not ultrafilter through collodion membranes of the type described and at the pressure indicated. Likewise, it does not dialyze through the cellophane membrane described.

It is well known that the spinal fluid of patients with marked hyperbilirubinemia and bilirubinuria does not contain bilirubin. Our own experience confirms this.

Our experiments show that serum bilirubin of patients with marked hyperbilirubinemia and bilirubinuria is entirely different in its filtrability through the human glomerulus and through collodion membranes. Its capacity to pass the spinal fluid barrier and through collodion and cellophane membranes appears to be the same.

CONCLUSIONS

Neither the bilirubin of the serum nor that of the urine of patients with obstructive jaundice can be ultrafiltered or dialyzed under the experimental conditions described. This is also true of bilirubin which has passed through the liver cells.

It appears, therefore, that the previous report of the "unvarying consistency in the behavior of bilirubin toward the collodion dialyzer and toward the renal filter" is untenable.

REFERENCES

1. Hoover, C. F., and Blankenhorn, M. A.: Dissociated Jaundice, Arch. Int. Med. **18**: 289, 1916.
2. Greenberg, D. M., and Gunther, L. On Determination of Diffusible and Non-diffusible Serum Calcium, J. Biol. Chem. **85**: 491, 1930.
3. Gregory, R., and Andersch, M.: Filterable Calcium of Blood Serum; Comparison of Filterable Calcium of Serum and Total Calcium of Cerebrospinal Fluid in Normal, Hyperparathyroid, and Hypoparathyroid States, Am. J. M. Sc. **191**: 263, 1936.
4. Gibson, R. B., and Goodrich, G. E.: Determination of Plasma Bilirubin; Modified van den Bergh Procedure, Proc. Soc. Exper. Biol. & Med. **31**: 413, 1934.
5. Thannhauser, J. A., and Anderson, E.: Methodik der quantitativen Bilirubin Bestimmung im Menschlichen Serum, Deutsche Arch. f. Klin. Med. **137**: 179, 1921.
6. Newman, C. E.: Bilirubin and van den Bergh Reaction, Brit. J. Exper. Path. **9**: 112, 1928.
7. Blankenhorn, M. A.: Acholuric Jaundice, Arch. Int. Med. **27**: 131, 1921.
8. Leschke, E.: Fortsetzung der Aussprache über Ikterus, Berl. Klin. Wchnschr. **58**: 848, 1921.
9. Barron, E. S. G.: Bilirubinemia, Medicine **10**: 77, 1931.

THE SO-CALLED "FIRST ACID CHANGE" IN FRESHLY SHED BLOOD*

B. S. Platt, M.B., Ch.B., M.Sc., Ph.D., Shanghai, China

A CHANGE of pH of from 0.02 to 0.095 units occurring in blood freshly removed from the body was described by Havard and Kerridge (1929), using the glass electrode, and confirmed by Laug (1931) by hydrogen and quinhydrone electrode measurements. In a study by Platt and Dickinson (1933) of the conditions required for accurate measurements by the glass electrode, it was found that temperature differences arising out of manipulations or due to factors in the design of the electrode system could give rise to potential differences which might be interpreted as changes, in the solutions examined, of hydrogen ion concentration—acid or alkaline according to conditions. These results, to us, afforded an explanation of the "acid change" of Havard and Kerridge, and it was further stated that no experimental evidence had been obtained of an acid change in blood of the type described by these writers. Other observations (Dickinson, Havard and Platt, 1933), in which some of the measurements were made by Havard, using the Stadie form of glass electrode, confirmed this view. It was also shown that, with blood on one side of the membrane, apparent acid or alkaline changes follow temperature differences according as a phosphate buffer solution of pH 7.0 or N/10 HCl was used on the other side of the membrane.

The first acid change was investigated by Laug (1934), who, by eliminating the possible artefact due to temperature differences, maintains that there is an acid shift in freshly shed blood, and that this acidity is probably the result of glycolysis preventable by the addition of potassium fluoride in comparatively large amounts. Recently Ferguson and DuBois (1936) have taken up this question. They state that Laug "gave good arguments against the possibility that the acid shift was a mere 'temperature artefact' as suggested by Platt and Dickinson." They feel that the artefacts discussed by Laug may be ruled out in their experiments. "The present data," they write, "agree with those of Laug and of Havard and Kerridge in identifying a true 'acid-shift' in shed blood."

Sifting the evidence, in view of the statements of Ferguson and DuBois, reveals, however, several inconsistences in the published data and the conclusions drawn from them. The present situation has arisen mainly out of confusion of the "first acid change" as originally defined and the acidity developed in blood as a result of glycolysis. In this note the evidence which has accumulated in support of the contention of Platt and Dickinson that the first acid change was in artefact is presented and the extent to which changes in acidity occur in freshly shed blood is indicated.

The main criteria of the first acid change as described by Havard and Kerridge are: that it is unaffected by sodium fluoride, that it takes place

*Received for publication, November 19, 1936.

within about six minutes at 38° C., and that it has a mean value of 0.05 pH. Laug (1934) himself states that well-defined acid changes proved to be due to nothing more than mere temperature artefacts traceable to warming up. The values he gives for the acid shift, for example, range from 0.05 pH units in about thirty-five minutes (as shown in his Fig. 1) to an average of 0.015 pH in twenty minutes for human blood as shown in Table II. The results of Ferguson and DuBois show (as shown in their Table I) only very slight changes in acidity and in one case an alkaline change in the course of twenty to thirty minutes during the clotting of rabbit's blood. The experiments in which significant changes in pH were observed were of several hours' duration and were carried out without the addition of fluoride.

Yoshimura (1935), in two of three experiments with fluorized human blood, found only a slow alkaline change beginning one hour after shedding; in the third experiment an acid change set in after about six minutes, continued for two hours, and was followed by an alkaline drift. He concludes from his work that the existence of the first acid change cannot be confirmed; that such acid change as is found in fresh blood is continuous and is due to glycolysis which is inhibited by sodium fluoride. This substance is, however, held responsible for an acid change due to an effect on the corpuscular membrane.

Harris, Rubin, and Shutt (1934) also agree with our finding that there is no acid change of the type found by Havard and Kerridge.

Recent careful work by Haugaard and Lundsteen (1936), using an electrode arrangement similar to that of Platt and Dickinson, is concerned more particularly with the relation between the change of pH of blood immediately after its removal from the body and the decrease of sugar. They have shown that the development of acidity follows closely the disappearance of sugar. During the first ten minutes after shedding, the change is no more than 0.006 pH. So small is the change of pH in their experiments that they consider the error of measurements made within about five minutes of obtaining the blood as being within the limits of experimental error for pH measurements. They appear to dispense entirely with anti-enzymatic substances.

The original experiments we made on blood were carried out in the presence of sodium fluoride and potassium oxalate. Not more than 10 c.c. of blood were withdrawn into a syringe containing 1 c.c. of 3 per cent potassium oxalate and 2 per cent sodium fluoride (neutralized); the same technique is described in detail by Dickinson and Havard (1933), who used it to determine the pH of arterial blood in cancer.

It must be conceded from the evidence here presented that there is no "first acid change" in freshly shed blood. The change originally described must have been a mere "temperature artefact." In the absence of fluoride, however, an acid change occurs which is part of a continuous glycolytic process. Even this reaction, according to the majority of workers, does not so rapidly result in alterations of acidity of the magnitude described by Havard and Kerridge. Careful examination of the published data goes to show that measurements made within five minutes of withdrawal from the body, without the addition of anticoagulants or anti-enzymatic substances,

but with proper temperature control and precautions to prevent loss of CO_2, yield values for the pH of blood which are within the accepted limits of accuracy for the measurement of pH in general (i.e., 0.01 to 0.02 pH units).

REFERENCES

1. Dickinson, S., and Havard, R. E.: The Reaction of the Arterial Blood in Cancer ,Brit. J. Exper. Path. **14**: 394, 1933.
2. Dickinson, S., Havard, R. E., and Platt, B. S.: The Measurement of the Hydrogen Ion Concentration of Blood by the Glass Electrode, J. Physiol. **78**: 28, 1933.
3. Ferguson, J. H., and DuBois, D.: Observations on the pH of Clotting and Citrated Blood, J. Lab. & Clin. Med. **21**: 663, 1936.
4. Harris, I., Rubin, E. L., and Shutt, W. J.: Modifications in the Use of the Glass Electrode for the Determination of the pH of Venous Blood, J. Physiol. **81**: 147, 1934.
5. Haugaard, G., and Lundsteen, E.: The Measurement of pH in Blood by Means of the Glass Electrode, Compt. rend. Carlsberg **20**: 1, 1936.
6. Havard, R. E., and Kerridge, P. T.: An Immediate Acid Change in Shed Blood, Biochem. J. **23**: 600, 1929.
7. Laug, E. P.: The Application of the Quinhydrone Electrode to the Determination of the pH of Serum and Plasma, J. Biol. Chem. **88**: 551, 1930.
8. Laug, E. P.: A Re-investigation of the Phenomenon of a First Acid Change in Whole Blood, J. Biol. Chem. **106**: 161, 1934.
9. Platt, B. S., and Dickinson, S.: The Technique of Electrode Measurements, Biochem. J. **27**: 1069, 1933.
10. Yoshimura, H.: On the Acid Change in Shed Blood, J. Biochem. **21**: 335, 1935.

SUPRARENAL TUMOR WITH PAROXYSMAL HYPERTENSION*

Case Report

Vernon L. Evans, M.D., Aurora, Ill.

IN RECENT years there have been reported several cases of tumor of the adrenal medulla or other chromaffin tissues, associated with paroxysmal attacks of hypertension and other manifestations of sympatheticotonia. The first classical description was by L'abbe, Tinel and Doumer, in 1922. C. H. Mayo, in 1927, reported the successful removal of one of these tumors with clinical cure of the patient. Belt and Powell have recently presented a case with a review of the literature. I wish to present another case of this type.

CASE REPORT

The patient was a girl twelve years old, when first seen, in March. Her illness began with what was said to be mumps of the ovaries four years previously. At that time she had much pain in the lower abdominal quadrants and was in bed for a few weeks. After that, the patient developed a ravenous appetite, profuse sweating, and purplish discoloration of the skin of the hands and feet. Two years ago, a basal metabolic rate determination was said to have given a figure of plus 60. For the past year or so there had been attacks of severe pectoral pain, accompanied by profuse sweating and discoloration of the skin. The severity and frequency of the attacks had been increasing.

When first seen, the patient looked to be of about the stated age, and was quite well developed and nourished. There was beginning development of the breasts and a small

*Received for publication, December 21, 1936.

amount of pubic hair. Menstruation had not occurred. The skin of the lower arms and legs was thickened and roughened and had a dusky red hue. The face was also affected in this way, but in a lesser degree. The pupils were equal and of medium size, and reacted promptly to light. The thyroid gland was normal to palpation. Over the cardiac apex could be heard a rather harsh systolic murmur, which was not transmitted. There was no cardiac enlargement to percussion. There was slight peripheral arteriosclerosis, as revealed by palpation of the brachial arteries. Examination of the ocular fundi revealed cotton-wool exudates in both macular regions with narrowing and some tortuosity of the retinal arterioles. The optic disks were swollen one or two diopters. No fresh hemorrhages were seen. Otherwise the physical examination revealed nothing noteworthy.

The laboratory data were as follows: Hemoglobin 88 per cent (Dare), erythrocytes 5,400,000, and leucocytes 14,200. The differential count revealed 71 per cent polymorphonuclear leucocytes, 28 per cent lymphocytes, and 1 per cent monocytes. The urinalysis was negative except for a trace of albumin (voided specimen). The Kline and Kolmer tests were negative. The phenolsulphonephthalein excretion was 65 per cent two hours after its intravenous injection. The blood urea was 50 mg. Roentgenograms of the chest and kidney areas revealed no abnormalities.

During the time the patient was under observation, a few attacks were observed. At the onset there would be a feeling of generalized weakness. After a few minutes there would

Fig. 1.—Photograph of suprarenal tumor removed at autopsy.

be a very painful sense of constriction in the substernal and precordial areas, the hands and feet would become blue and cold and there woulld be profuse generalized sweating. At the end of the attack there would be a frontal headache, tinnitus aurium, and often repeated vomiting. The duration of the attacks varied from a few minutes to two hours. The paroxysms would take place at any time of the day, but most often in the morning, shortly after breakfast. The patient was unable to induce attacks by twisting or bending the body, as was the case in the patient reported by Porter and Porter.

During the attack, the blood pressure rose to as high as 285 systolic and 230 diastolic, and the heart rate became very rapid. The pupils were widely dilated. The retinal arterioles were so narrowed as to be scarcely visible. A blood sugar determination was made in an attack and the figure was 153 mg. per cent. (This was a few minutes after breakfast.)

After some discussion with the parents of the patient, she was finally hospitalized on May 14 for operative exploration of the adrenal glands. At that time there was a fever, ranging from 98.4 to 100.0°, and there was continuous sweating. Operation was scheduled for the morning of May 16, but as the patient developed some pectoral pain and an elevation of blood pressure to 185 systolic and 130 diastolic, the operation was deferred. These minor attacks continued daily, necessitating further postponement of the operation. On May 19 there was a very severe attack followed by symptoms and signs of shock. The blood pressure dropped to 30 and the pupils became very small. The heart rate was approximately 200.

One-fourth cubic centimeter of adrenalin was given, and in a few minutes there was much improvement. The next day there was another severe attack followed by shock. Adrenalin was again administered, but before other shock therapy could be instituted, the patient died, the respiration continuing for a few seconds after the heart had stopped.

Autopsy.—The livid discoloration was replaced by the usual postmortem pallor. There was no free fluid in the thoracic cavity and the lungs showed no signs of pulmonary edema or other abnormality. The heart appeared normal in size. Examination of the endocardium and valves revealed no abnormalities. The myocardium appeared normal on cut section. On opening the coronary arteries, there was found no evidence of arteriosclerosis other than a few small scattered atheromatous plaques. The aorta also contained only a few similar plaques. On exploring the left renal area there was found a tumor about the size of a hen's egg. This was situated anterior to and above the kidney retroperitoneally, with the tail of the pancreas around it. The adrenal gland was intimately associated with the tumor, about one-half of its substance projecting from its surface. The mass shelled out quite readily, but there was some adherence to the peritoneum. The tumor was rather soft, and on opening it, was found to be composed of pliable glandular tissue with some cystic degeneration and gelatinous formation in the center. The right adrenal gland appeared grossly normal. Both kidneys appeared normal, as did the stomach, intestine, liver, and spleen. There were many enlarged lymph glands in the mesentery.

The specimen was sent to Dr. Donald Beaver of the Department of Pathology of the Mayo Clinic, who reported the tumor to be composed of adrenal medullary tissue, a pheochromocytoma.

COMMENT

The case is interesting from many viewpoints. Could surgical aid have been secured early enough, the patient might have been cured. While the operation is a hazardous one, as has been found in previous cases, the tumor showed no signs of malignancy and its removal would have induced permanent relief from the attacks.

The clinical picture was quite like the other reported cases, but the death was different. Of the other cases that terminated fatally, that of L'abbe, Tinel and Doumer died of pulmonary edema. Those of Rabin and Vaquez, Danzelot and Gerandel died in coma and that of L'abbe, Violle and Azerod died of cerebral hemorrhage. In the case reported here the patient was perfectly conscious a few minutes before death, and at autopsy there were found no signs of pulmonary edema. Death was apparently due to shock, or a condition closely simulating it.

In all of the cases in which the tumors were removed, severe shock was a postoperative complication and the patient of Belt and Powell died of postoperative shock. This might lead one to hazard a theory. It would appear that the body has some mechanism of defense against the abnormally large amounts of adrenalin produced by the tumor. Perhaps this defense mechanism, be it an "anti-hormone," as suggested by Collip, or the breaking-down of adrenalin by the liver or other organs, becomes suddenly too powerful when the tumor is removed, with the development of shock (antithesis of the condition occurring in the attacks).

Why, in all cases reported, the syndrome should be of such a paroxysmal nature is problematical. In the Porters' case the patient could produce attacks at will by twisting the body into certain positions. It would appear that mechanical pressure on the tumor might have caused the expulsion of adrenalin into the circulation. In the case above reported, as well as in several of the previous

ones, the attack would occur for no apparent reason. However, there was a great tendency for the attacks to occur shortly after meals and especially after breakfast. This would suggest that some of the digestive organs played a part in maintaining this hypothetical balance between the adrenals and some other organ.

Unfortunately the thyroid gland was not removed at autopsy. Among the symptoms presented by the patient, there were many that could have been interpreted as due to hyperthyroidism, as well as hypersuprarenalism. However, there was no stare, tremor, nor history of recent weight loss. Determination of the basal metabolic rate was not attempted, because the patient could not be gotten into a condition anything like "basal."

CONCLUSIONS

1. A case of suprarenal medullary tumor (pheochromocytoma) has been presented with autopsy findings.

REFERENCES

1. Belt, A. E., and Powell, T. O.: Clinical Manifestations of the Chromaffin Cell Tumors Arising From the Suprarenal Medulla, Surg. Gynec. Obst. **59**: 9, 1934.
2. Collip, J. B.: Inhibitory Hormones and the Principle of Inverse Response, Ann. Int. Med. **8**: 10, 1934.
3. L'abbe, M., Tinel, and Doumer: Crises solaires et hypertension paroxystique en rapport avec une tumor surrenals, Bull. et mém. Soc. med. d. hôp. de Paris **46**: 982, 1922.
4. L'abbe, M., Violle, P. L., and Azerod, E.: L'adenome medullaire surrenal avec hypertension paroxystique, Presse méd. **38**: 553, 1930.
5. Mayo, C. H.: Paroxysmal Hypertension With Tumor of Retroperitoneal Nerve, J. A. M. A. **89**: 1047, 1927.
6. Porter, M. F., and Porter, M. F., Jr.: Report of Case of Paroxysmal Hypertension Cured by Removal of Adrenal Tumor, Surg. Gynec. Obst. **50**: 160, 1930.
7. Rabin, C. B.: Chromaffin Cell Tumor of Suprarenal Medulla (Pheochromocytoma), Arch. Path. **7**: 226, 1929.
8. Vaquez, H., Danzelot, E., and Gerandel, E.: Le Surrenalome hypertensie, Presse méd. **37**: 169, 1929.

33 South Island Avenue

A SUPPORTING, ELASTIC BELT FOR USE IN ABDOMINAL OBESITY (POSTURAL SYNDROME)*

WM. J. KERR, M.D., AND JOHN B. LAGEN, M.D., SAN FRANCISCO, CALIF.

MANY belts have been devised and are used routinely for abdominal support. Their main purpose is to correct either obesity or ptosis of the abdomen by lifting and holding in the abdominal wall. They accomplish this by supplementing or replacing the abdominal musculature which has either lost its tone or, more usually, has become flabby and no longer functions properly. These belts are constructed in many ways, but all have similar materials and serve the same purpose. We have a collection of inadequate belts donated by patients, and these show great variation in type.

We have devised a belt for use on patients suffering from symptoms due to the effects of a pendulous abodmen. Obesity, most frequently generalized but occasionally limited to the abdomen, is usually the precipitating factor. The result, when the condition has reached the point where the abdominal muscles can no longer sustain the added weight, is a heavy, pendulous belly. The condition frequently results in a train of symptoms which we have termed the "postural syndrome."[1] There is no doubt that any individual with abdominal ptosis, whatever its cause, will have symptoms and signs when the condition has been present long enough. The correction of the condition in these patients depends upon loss of weight, restoration of muscular function, and postural exercises. A proper supporting belt is a distinct aid in treatment, in the early months especially.

The belt that we are presenting embodies several new principles. As a whole it is not unusual, or very different from the many we have seen. However, it is constructed with the physiologic function of the abdominal wall in mind, and is designed to supplement and aid the ventral muscles rather than to replace them. A tightly wound cloth binder would support the abdomen more efficiently but would not permit mobility necessary for respiration.

Fig. 1, *C* shows the belt in place on a patient. Fig. 1, *B* shows some of the details of construction. The supporter is made of coutil, pekin-stripe cloth and elastic goring; it is fastened on by skate buckles and hooks.

The front sections and the back section are of double thickness. The outer layer is of high quality coutil, the inner of pekin-stripe cloth. The only difference between the two is in the softer and finer quality of the pekin-stripe material which, being next to the skin, prevents chafing. Both sections are tailored or fashioned. There are three double stays in the back section with a seam at each, which permits fashioning the supporter to fit the contours of the individual patient. The back section is 8½ inches in height, and the stays are of whale-

*From the Department of Medicine, University of California Medical School.
Received for publication, December 28, 1936.
Assisted in part by a grant from the Christine Breon Fund.

bone. For conditions of extreme lordosis, the cloth section in back may be extended up to the twelfth thoracic vertebra, and have incorporated in it firm duraluminum or steel stays. The stays should not, however, be bent to fit the curve of the lumbar spine but should touch the body at only the upper and lower edges of the belt, allowing the middle to span or bridge the lordosed spine.

The left front section, Fig. 1, *C* (*1*), has the hooks attached to it. The right front section, Fig. 1, *B* (*1*), has attached to it the straps carrying the buckles. The height of the front from top to bottom varies somewhat according to the circumference. For patients 38 inches in circumference at the crest of the ilium,

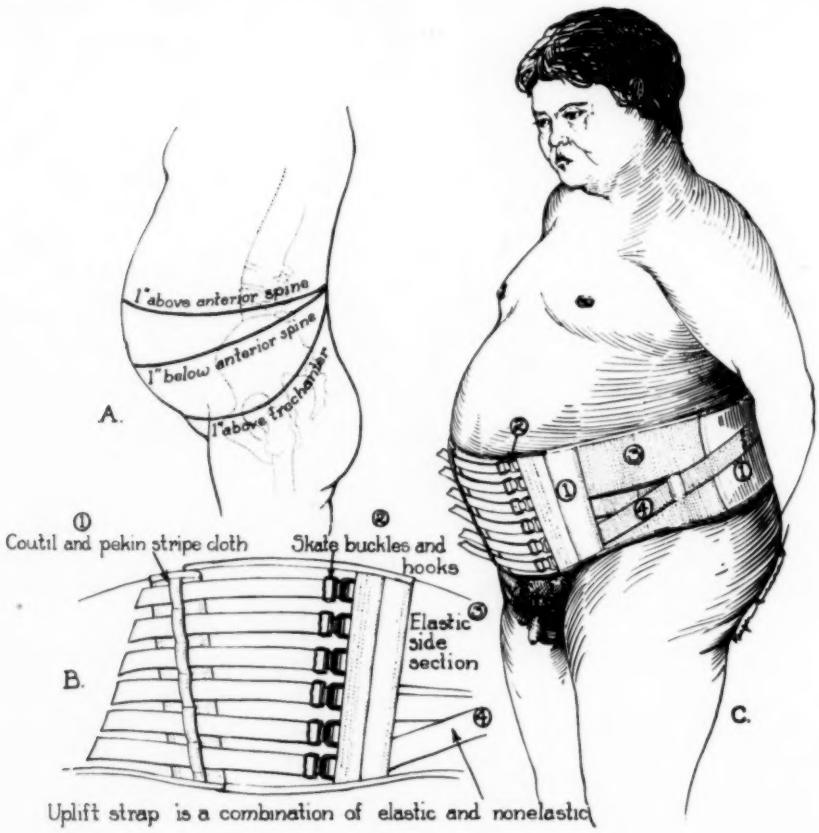


Fig. 1.

a height of 9 inches is usually most satisfactory; if the patient is exceptionally tall, a front height of 10 inches can be used to advantage; for patients of a circumference of 44 inches or more, a height of 10 to 12 inches is usually required. The left front section extends across the abdomen as a flap 6 inches long, padded between the two layers with strips of daisy cloth. This provides a soft base for the straps to lie on. The width of each half of the cloth section across the front is considerably less than in the usual supporter, being an average of 2½ inches wide. The total width of the nonelastic front is about 5 to 7 inches, but this varies with the tightness of the belt. The front flap slips under the right side of the belt as it is tightened.

The elastic lateral sections, Fig. 1, *C* (*3*), each are made of two pieces of firm elastic goring 6 inches wide and approximately 7 inches long, placed one above the other. The two pieces overlap about 2 inches in the center, which gives greater support to the front center. The lower piece of goring on either side is set diagonally downward toward the front, exerting therefore an upward lift rather than a straight posterior pull.

The uplift strap, Fig. 1, *C* (*4*), is a combination of elastic and nonelastic material. The front forked section consists of firm elastic webbing 1½ inches wide. The latter extends to a buckle located toward the top of the supporter just back of the hips, allowing tightening or loosening of the strap as desired. The uplift straps, being placed diagonally, exert a supporting rather than a suppressing pressure on the lower abdomen.

The belt is fastened in front by means of six skate buckles and hooks (Fig. 1, *B* (*2*). The buckles are slipped over the hooks with the straps loose; each may then be individually tightened to the desired degree. The extra length of strap is held up by slots at the origin, rather than allowed to dangle.

Fig. 1, *A* shows the measurements required in making up a belt when the patient is not able to be fitted personally. The measurement for the upper edge of the belt is taken around the body about 1 inch above the anterosuperior spine of the ilium. The measurement for the middle of the belt should pass over the largest part of the abdomen. The measurement for the lower edge should follow the lowest line of the abdomen in front, and cross the spine in back three or four inches below the upper measurement.

The dimensions of a size 40 belt are approximately as follows:

1. *Back:* 16½ inches long at the top and 18 inches at the bottom, with three double pieces of whalebone 4½ inches apart. Height is 8½ inches.

2. *Elastic side sections:* 6 inches long, made of two pieces of goring 5 inches wide with 2½ inches' overlap, giving a height of 8 inches.

3. *Uplift strap,* the elastic arising at the bottom in front: 5 inches long, continued by a web strap 6½ inches long, passing through a tightening buckle and inserted into the beginning of the back piece 2 inches below the top.

4. *Front sections:* 2½ inches wide and 9½ inches high, the tongue 6 inches long.

It should be stated that these measurements do not designate the contouring necessary for proper fitting to the body; nor do they take into account the extra length required for seams, etc. They were taken from a finished product, laid flat.

Patients are taught to put on the belt before arising in the morning, preferably outside the undershirt. If a union suit is worn, the belt can be worn underneath it over a thin garment. The patient should be in the supine position when putting on the belt, and the belt should be tightened from below upward. This is important in order to move the abdominal fat and viscera upward, rather than to compress it in the lower abdomen.

The unusual feature of the belt is the width and height of the elastic side sections, particularly the width. This is obtained by making the front narrow,

permitting the elastic side sections to extend farther forward on the abdomen where the elasticity is much more beneficial than if it were placed toward the back. This increased elasticity permits expansion of the belt during inspiration. This aids rather than suppresses abdominal breathing, and prevents limiting respiration to the thorax, as is seen in obese states naturally or where a firm, inelastic belt is applied. The increased elasticity also aids in expiration, and overcomes the prolonged expiratory period seen in those patients with depressed diaphragms due to abdominal ptosis or emphysema. The diaphragm more readily assumes the expiratory position, and is ready to descend with the next inspiration. There is no decrease in the supportive effect during the inspiratory expansion.

These belts have been made for us by the C. H. Hittenberger Co., 1103 Market St., San Francisco. We wish to express our appreciation to this firm for helpful criticism in the original designing of the belt, and for courtesy and care in fitting our patients.

REFERENCE

1. Kerr, Wm. J., and Lagen, John B.: The Postural Syndrome Related to Obesity Leading to Postural Emphysema and Cardiorespiratory Failure, Ann. Int. Med. **10**: 569, 1936.

THE TOXICITY OF MORPHINE SULPHATE AND THE PRESSOR EPISODES*

A. J. NEDZEL, M.D., CHICAGO, ILL.

IN A previous paper (Nedzel[1]), it has been shown that the Straub sign and the minimal lethal dose of morphine sulphate in mice vary from day to day. The animals used in our experiments were approximately of the same age, were kept under same conditions and had the same diets; the morphine used was of the same lot and, in experiments with minimal lethal dose, it was given proportionally to the individual weight of the animals, though such varied only a few grams.

With the modern concept of variability in physiologic state of the organism, intimately connected with constant changes in atmospheric conditions (Petersen[2]), we have to look in this direction in seeking the explanation of our findings.†

The first group of experiments consisted in producing a Straub sign in mice by injecting a small dose of morphine sulphate (0.4 mg.). The mice were kept on our standard diet (Nedzel[1]). For seven weeks (five days a week), ten mice received morphine injections daily.

Graph 1 presents the results obtained in these observations. The upper broken line designates the positive Straub sign in 9 or 10 mice obtained on the given date. On the left is shown time (in hours) of duration of the Straub sign from the moment of morphine injection. Below this broken line the wide, uneven, black line, with unshaded line in the center expresses the official temperature on given days. The upper margin gives daily maximum temperature, the lower, the minimum temperature. The middle unshaded line presents the mean temperature. The lower curve is a barograph, giving the official barometric pressure for the days on which the experiments were performed.

The duration of maximal reaction (Straub sign) with our dose of morphine should, according to Herrmann,[3] be 2¾ hours.

In our findings we see great variations in the duration of the Straub sign. As the graph shows, this reaction on certain days becomes shortened greatly, and

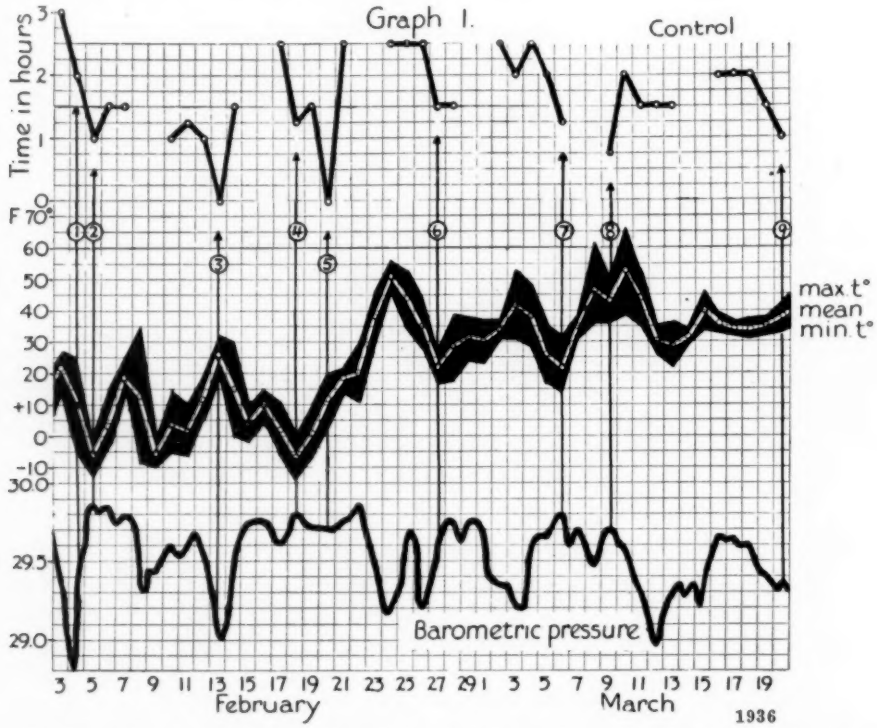
*From the Department of Pathology and Bacteriology and the Department of Pharmacology, University of Illinois, College of Medicine.

Received for publication, December 29, 1936.

†A pressor episode, a pressor crisis (Pal crisis), the ARS phase of Petersen, Hochspannungskriese of the Germans, represents a period of relative sympathicotonia when, with adrenal and pituitary preponderance, both systolic and diastolic blood pressure are increased. At the same time there is usually an increase in the blood pH and a decrease in the CO^2 content. During this period there is a tendency to contraction of the smaller vessels and capillaries and with this a relative anoxia may exist in the various regions. Following this phase a corrective phase sets in which is initiatel by the accumulation of acids produced by anoxia, by capillary active substances, by an increase in thyroid secretion, etc. Constant pendulation of pressor crests with corrective phases of vascular dilatation are commonly initiated by change in the atmospheric circulation in our latitude, but, needless to state, a multitude of intercurrent events (psychic, physical activity, trauma, etc.) may bring about similar though usually transient episodes of this character.

if we draw vertical lines connecting the line representing the data of our findings with the lines of temperature and barometric pressure, we may establish a definite connection. These lines are numbered.

At lines 1 and 2 we record a drop in duration of a maximal Straub sign and this is connected with beginning of a pressor episode (polar front), the temperature drops and barometric pressure rises. At No. 3 we see somewhat the reverse condition, the temperature going up with a deep fall in barometric pressure. But if we look at the meteorographs of a few previous days, then we observe that the animals just went through a considerable pressor episode, are fatigued, which makes them unable to recover properly (Petersen[2]). We ob-



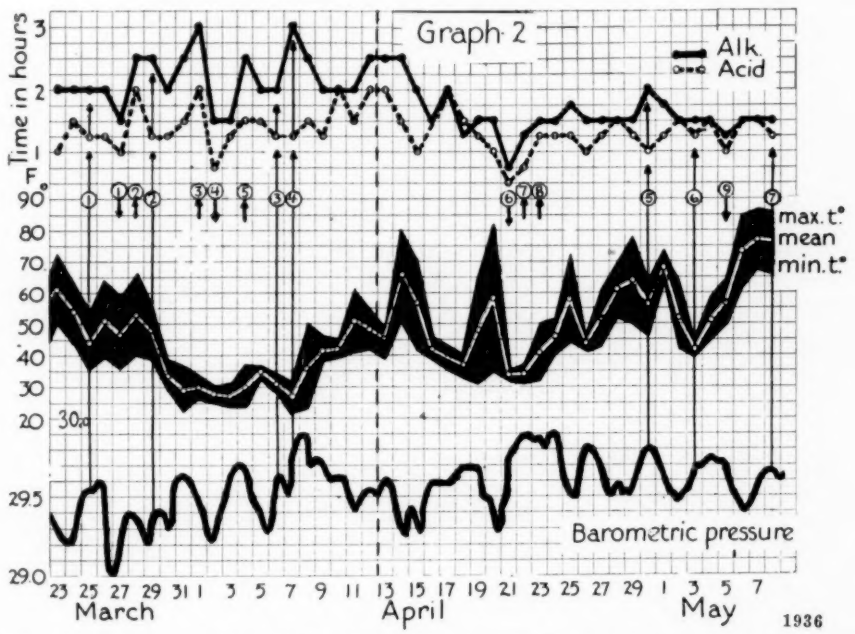
Graph 1.—The upper broken line designates the positive Straub sign in nine or ten mice obtained on the given date. On the left is shown time (in hours) of the duration of the Straub sign from the moment of morphine injection. The wide, uneven, black line expresses the official temperature (maximum, mean, and minimum) on given days. The lower curve is a barograph, showing the official barometric pressure for the days on which the experiments were performed. The figures below give dates on which experiments were done.

served a somewhat similar reaction at 5; but at 4, 6, 7, 8 and 9, the shortening of a time of maximal Straub sign coincides obviously with the pressor episodes.

Graph 2 presents our findings in a second series of experiments, where we have used two groups of mice, using ten animals from each group daily for forty-seven consecutive days. In the first group, the animals were given water with 2 per cent of sodium citrate which raised the pH of their urine up to 8.0 (normally, with our diet, it was around 6.0), and in the second group, the animals received water with $\frac{1}{30}$ per cent of hydrochloric acid which lowered the pH of their urine to 5.5. The upper black line of Graph 2 presents the findings in

animals which were on water with added sodium citrate, their urine reaction being alkaline, and the interrupted line presents the findings in the group of mice that had water with hydrochloric acid and whose urine was constantly acid. In comparison with Graph 1, we have added here numbered arrows, pointing to the dates on which the prolonged or shortened Straub sign was observed simultaneously in both groups of animals. All other details are the same as on Graph 1.

We observe here, that the animals kept on water with hydrochloric acid showed, practically throughout the forty-seven days, a shortened duration of Straub sign. The vertical interrupted line drawn between 12 and 13 of April roughly divides all the observations in two parts. In the first part, where we see gradual, relatively slow changes in temperature, the duration of Straub



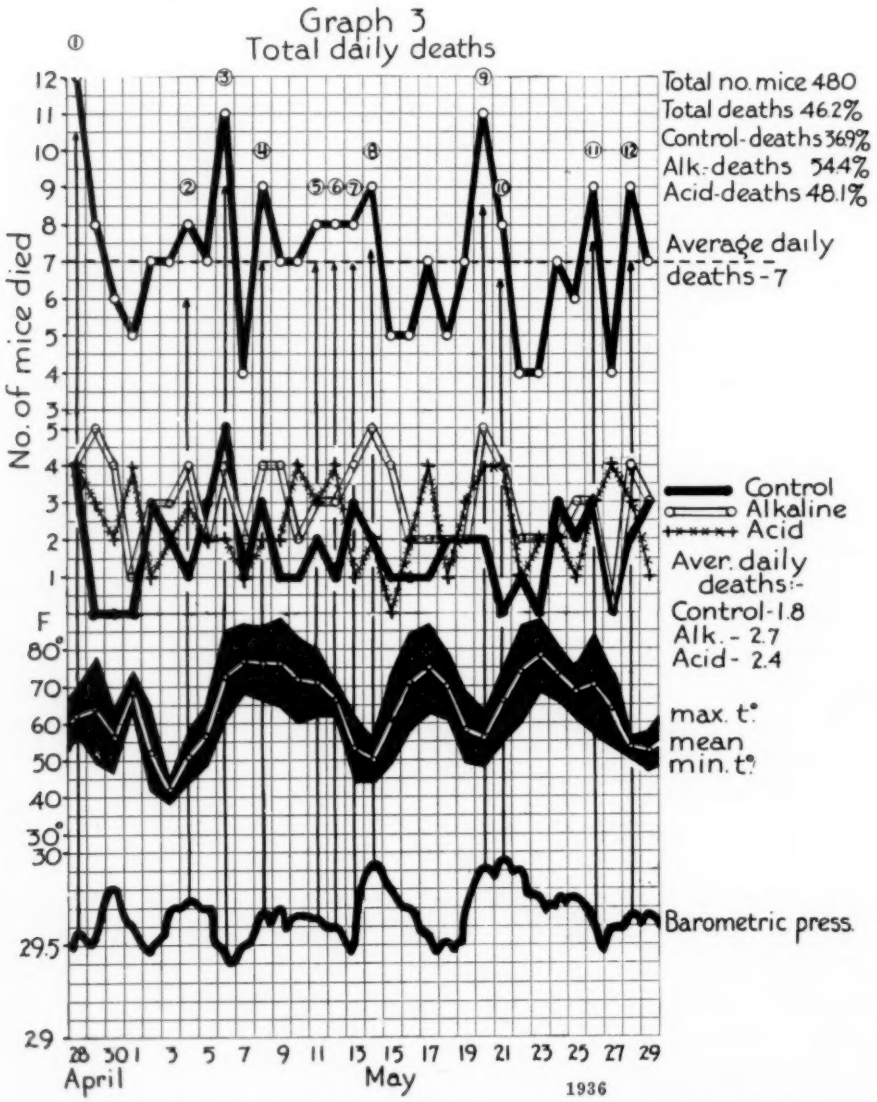
Graph 2.—The upper black line designates the positive Straub sign in animals on alkaline diet and the interrupted line presents the findings in the mice on an acid diet. All other details are the same as on Graph 1.

sign in both groups is longer than in the second part, and the mice with alkaline urine show a Straub sign considerably longer than that of the mice on hydrochloric acid. In the second part, where the temperature changes show greater variations, the length of the maximal Straub sign varies little in the two groups and is noticeably shorter in comparison with the first part of the experiment.

The vertical lines (which all, from 1 to 7, fall on the polar front) 1, 2, 3, 6, and 7 point out dates on which the alkaline mice withstood the pressor episodes and did not show any change in Straub sign, while the acid animals became less irritable and the duration of Straub sign in them was shortened. Vertical line 4 presents the date when there was a well-pronounced pressor episode and the acid animals retained their short duration of the Straub sign of the previous

day, but alkaline animals definitely had prolonged duration of this sign. Vertical 5, which falls also on a pronounced polar infall, points to the prolongation of the sign in alkaline mice and shortening of it in the acid animals.

Arrows 1, 4, and 6, when the duration of Straub sign has been shortened in both groups, point to dates with beginning pressor episodes. Arrow 9, when



Graph 3.—The upper black line presents the total deaths of mice of all three groups. The figures on the left show the number of dead animals of the total number of fifteen. The three lines beneath the upper black line express the daily deaths of mice from each group. The black line represents the deaths in control group; the double line, alkaline group; the cross-hatched line, the acid group. The figures on the left show the numbers of mice that died. All other details are the same as on Graphs 1 and 2.

the duration of the sign in both groups was also shortened, points to date with rising temperature, but the barometer is still high, just at the end of the pressor episode that happened on previous days. Arrows 2, 3, 5, 7, and 8 point to

prolonged duration of the Straub sign in both groups. On all these dates the pressor episodes have passed and either the temperature was rising or barometric pressure was declining.

The third series of experiments was on mice to which we have administered a minimal lethal dose (5 mg. per 20 gm. of weight) of morphine sulphate. The animals were divided into three groups. First group, controls; second, with sodium citrate in water; and third, hydrochloric acid in water. Daily, for thirty-two consecutive days, to five mice of each group has been administered the mentioned dose of morphine sulphate. The findings are presented in Graph 3. The upper line presents the total deaths of mice of all three groups by days. The figures on the left show the number of dead animals of the total number of fifteen. The interrupted horizontal line at figure seven designates the average daily deaths, which has been seven. The three lines beneath express the daily death of mice from each group. The black line represents the deaths in the control group; the double line, alkaline group; and the cross-hatched line, the acid group. The figures on the left show the number of mice that died. The lower lines give meteorologic data (temperature and barometric pressure) on the designated dates. The vertical lines point to peaks, where the average death rate of all three groups has been above the number of average daily deaths (seven).

The verticals 1, 2, 4, 7, 8, 9, 10, and 12 definitely connect the high death rate on corresponding dates with a pressor episode. Verticals 3 and 11 fall on the days with a sudden rise in temperature and falling barometer just after the pressor episode. Vertical lines 5 and 6 fall on slightly increased number of daily deaths, which coincide with a very slight falling barometric pressure, but here we had also a definitely falling temperature. The lowest death rates, as can be seen on the graph, coincide with moderate barometric pressure and high temperatures.

The lower lines, representing daily deaths in three groups of mice separately, point out unquestionably the daily variations in each group and these variations must be connected with the daily variations in meteorologic conditions, not only for vast clinical and experimental evidence (Petersen[2]), but also for lack of any other possible explanation. We consider that the number of animals used daily from each group (five) is inadequate for drawing definite and detailed conclusions. Additional experiments are to follow.

It is of interest to mention here observations carried out by Macht.[4] He found that there is a very definite influence of barometric changes and other meteorologic conditions on the potency of digitalis for cats. He points out that the difference in the toxicity is due to changes in the physiologic functions of the cats (his experimental animals) and more particularly to changes produced in the respiration and circulation by the fall in the barometer. He concludes that his observations on digitalis and some other drugs point to the fact that fluctuations in the barometric pressure and other changes in atmospheric conditions may play an important rôle in the action of various drugs.

CONCLUSIONS

1. The duration of the Straub sign in mice is definitely connected with pressor episodes (polar fronts).

2. The mice on alkaline water give a Straub sign of greater duration in comparison with the animals on acid water.

3. The more changes in meteorologic conditions are pronounced, the greater is the duration of Straub sign in both groups of mice, on alkaline or acid diet.

4. The minimal lethal dose of morphine obviously increases the total death rate of all three groups of mice (on ordinary, on alkaline and on acid diet) on the days of pressor episodes.

5. Ordinary, alkaline or acid water in mice diets definitely changes their reaction toward injected minimal lethal dose of morphine sulphate.

I wish to express my sincere gratitude to Dr. Bernard Fantus for his many valuable suggestions.

REFERENCES

1. Nedzel, A. J.: Variations in the Toxicity of Morphine Sulphate, J. Lab. & Clin. Med. **22**: 1031, 1937.
2. Petersen, Wm. F.: The Patient and the Weather, Vol. I-IV, Ann Arbor, Michigan, 1934-36, Edwards Brothers.
3. Herrmann, O.: Biologische Nachweise des Morphins, Biochem. Ztschr. **39**: 216, 1912.
4. Macht, David J.: Discussion of Fenn's, S. K., and Gilbert's, N. C. paper: Anginal Pain as a Result of Administration of Digitalis, J. A. M. A. **98**: 103, 1932.

INFLUENCE OF DIET UPON THE ACTION OF PHENOBARBITAL SODIUM*

A. J. Nedzel, M.D., Chicago, Ill.

PREVIOUSLY we have reported (Nedzel[1, 2]) our observations on reaction of rabbits to cocaine and ethylhydrocupreine hydrochloric injections, the animals being on carrots, mixed, and oats and water diets. It was found that the rabbits on carrot or mixed diet reacted to the injection of cocaine more or less similarly; though the animals on carrot diet reacted more vigorously. The animals on oats and water diet were definitely more sensitive to cocaine poisoning, namely, the toxic effects were greater and recovery was considerably delayed.

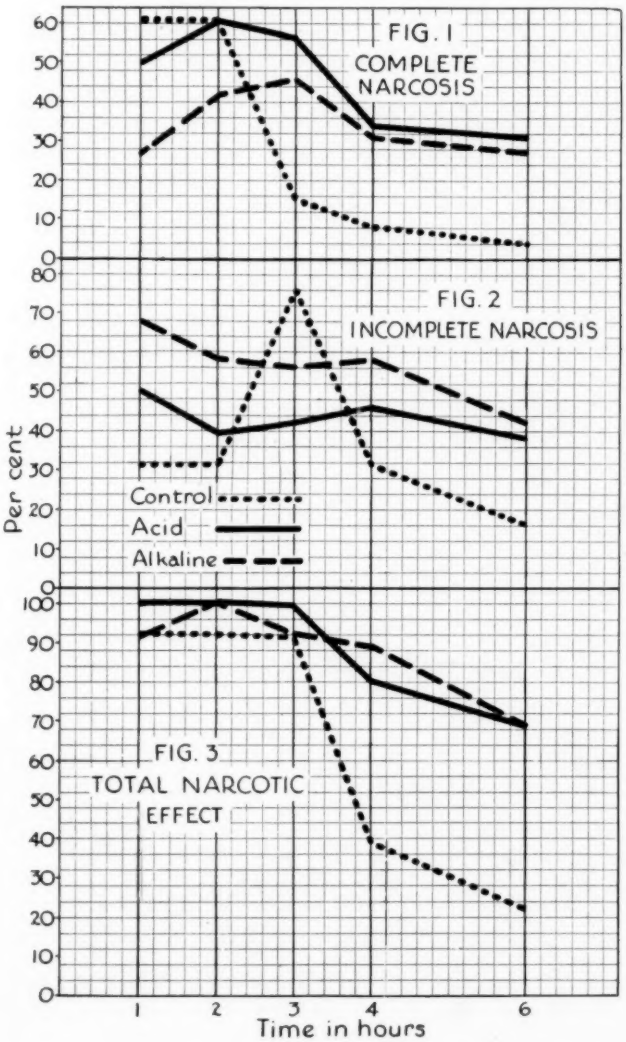
In experiments with ethylhydrocupreine injections animals on an oat diet reacted similarly, as in cocaine poisoning experiments; they appeared to be more sensitive to the ethylhydrocupreine than the animals on a mixed diet. But here, contrary to experiments on cocaine poisoning, the rabbits on carrot diet reacted less severely than the animals on mixed diet.

In continuation of studies described, we have undertaken another series of experiments, presented here, replacing cocaine and ethylhydrocupreine hydrochloric with phenobarbital sodium.

*From the Department of Pathology and Bacteriology and the Department of Pharmacology, University of Illinois, College of Medicine.

Received for publication, December 29, 1936.

The observations were conducted on 78 rabbits, divided into three groups, each group consisting of 26 animals. Each group was kept from fifteen to twenty days on the special diets. In the first group (mixed diet) the animals were fed oats, carrots, alfalfa, cabbage, and water (our standard normal diet); the second group was fed oats and water; the third, carrots exclusively. By means of Folin's method the reaction of urine of the animals was tested daily.



At the end of the first week the urine of the rabbits of the first group was slightly alkaline or acid, although generally more alkaline; the urine of the second group (oat diet) showed stable and distinct acid reaction (up to 90); and that of the third (carrot diet) group was persistently alkaline.

The experiments have been performed by injections of phenobarbital sodium into the marginal ear vein of a rabbit. The injections were performed uniformly, the duration of injection being about ten seconds. The dose of pheno-

barbital sodium was 0.08 gm. in 0.5 c.c. of water per kilogram of animal weight. The rabbits were observed every hour for six hours from the time of injection. Our findings are presented on accompanying chart.

The abscissa represents the time of observations in hours, and the ordinates, the percentage of animals which reacted positively. The chart is composed of three figures: Fig. 1 presents the percentage of animals in complete narcosis at given time, Fig. 2, incomplete narcosis, and Fig. 3, the total effect of phenobarbital sodium. The dotted line represents the control animals (mixed, standard diet), the broken line, the animals on carrots, and the solid line, oats and water group.

The lines (Fig. 1) show that in the control group the full narcosis was observed in 60 per cent of the animals, which lasted in great majority of animals for two hours. The alkaline group gave a lesser number of fully narcotized animals, and the narcosis appeared and disappeared more slowly. The acid group of rabbits reacted less at first, but the full narcosis remained longer and in larger number of animals.

Fig. 2 of the chart happens to appear as a supplement to the upper part. Here are recorded the findings in animals which did not undergo complete narcosis or were recovering from such and appeared either drowsy or reacted to the touch by opening of the eyes or by movements. The control animals recovered much faster than the animals on carrot or oat and water diets. The incomplete narcosis seems to be more pronounced in the group of animals on carrot diet in comparison with the group on oats and water. In twenty-four hours all animals have recovered. Fig. 3 presents the total effect of the phenobarbital sodium in the experimental animals (Figs. 1 and 2). This total effect appears in the acid group in a larger per cent than in two others. In the alkaline group we observe that the total effect is less than in the first group, fades slower in a four- to six-hour interval after the beginning of the experiment. At six hours the effect is the same as in the acid group. The animals in a mixed diet show total effect less and its disappearance much earlier than in both other groups.

In forty-eight hours, 2 rabbits from control group were found dead and in twenty-four hours, one dead in alkaline group. The deaths were attributed to pneumonia.

CONCLUSIONS

1. A large number of animals on an oat diet undergo prolonged general narcosis from phenobarbital sodium.

2. A lesser number of animals on carrot diet undergo complete narcosis in comparison with the oat diet group.

3. Animals on mixed diet undergo complete narcosis more quickly, but the latter fades comparatively very soon.

I am greatly indebted to Dr. Bernard Fantus for his suggestions and advice.

REFERENCES

1. Nedzel, A. J.: Cocaine Poisoning as Influenced by Diet, J. LAB. & CLIN. MED. **19**: 874, 1934.
2. Nedzel, A. J.: Influence of Diet Upon the Toxicity of Ethylhydrocupreine Hydrochloride, J. LAB. & CLIN. MED. **20**: 944, 1935.

STUDIES RELATING TO THE TOXICITY OF FLUORINE COMPOUNDS*

C. A. KEMPF, M.S., D. A. GREENWOOD, M.S., AND VICTOR E. NELSON, M.S.,
AMES, IOWA

OUR knowledge concerning the distribution of fluorine in drinking waters and the effect of such waters on the structure of the teeth has been appreciably increased by the recent work of Smith, Lantz, and Smith,[1] Churchill,[2] McKay,[3] Kehr,[4] Ostrem, Nelson, Greenwood, and Wilhelm,[5] Boissevain,[6] Dean,[7] Sebrell, Dean, Elvove, and Breaux,[8] and Boruff and Abbott.[9] Smith, Lantz, and Smith[1] were the first to show that mottled enamel is due to fluorides in drinking waters; in fact, their analyses of waters from endemic areas show that the fluorine concentration in such waters is high.

In 1932 mottled enamel was first shown to be present in Iowa by Ostrem, Nelson, Greenwood, and Wilhelm.[5] They pointed out that in the city of Ankeny and surrounding territory there exists a high incidence of mottled enamel, and they correlated this condition with the fluoride concentration of the water of that region. Analyses of the city water supply of Ankeny show about 7.5 to 8.5 parts of fluorine present per million of water. It is now known that excess fluorides occur in waters of other communities in the State of Iowa, and in recent years there has been a considerable increase in the number of regions affected by such waters not only in this country but throughout the world; hence, the effects of fluorine upon the body system and the problems involved in its removal are of importance to medicine and allied sciences.

EXPERIMENTAL

It has been found from work in this laboratory and elsewhere that ingestion of fluorine waters or certain fluorides causes damage to the teeth, resulting in the production of the dystrophy called mottled enamel. Abnormal enamel is very prevalent in the region of Ankeny. All of the children reared in the community, whose water consumption has been limited to the general city supply, have mottled teeth. The effect is almost entirely confined to the permanent teeth, although a few cases of deciduous mottling have been observed. Children not born in the community may or may not be affected, depending upon their age when they arrived in the community; and the degree of mottling in such children will depend on the extent of calcification or formation of the teeth prior to their arrival, as is to be expected. That the tooth defect is due to fluorides was demonstrated by analyses and feeding experiments on rats. Analyses revealed fluorine to the extent of 7 to 12 parts per million in the waters of Ankeny and surrounding territory. The water containing fluorine comes from deep wells; shallow wells give water containing little or no fluorine. Where the people have access to deep and shallow wells the

*From the laboratories of Physiological Chemistry, Iowa State College.
Received for publication, December 30, 1936.

severity of the mottling is related to the amounts of each water consumed. Fig. 1 shows typical mottling of the teeth of a child from this region. Fig. 2 illustrates a section of a mottled human tooth.

Rats fed certain fluorides, such as NaF or fluoride water, in all cases developed mottled enamel very similar to that observed in man. Experiments

Fig. 1.—Showing the mottled condition of the permanent teeth of a child.

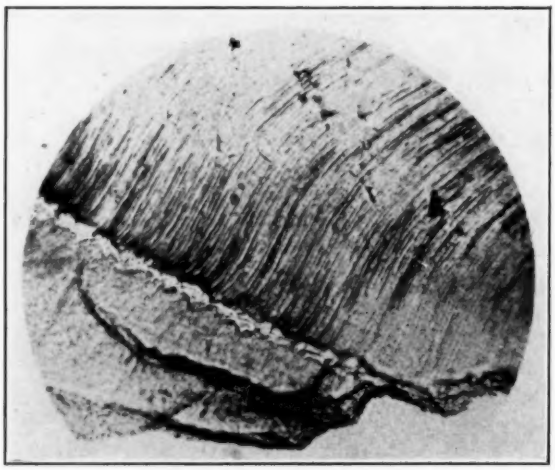


Fig. 2.—Showing section of human mottled tooth.

were made on dogs also, in order to ascertain whether fluorides or fluoride water would produce like results and whether or not the deciduous teeth would be affected. The dog differs from the rat in that it has both deciduous and permanent teeth; whereas, the rat has one set of teeth which, because of a persistent pulp, continue to grow during the life of the animal. The results of these experiments demonstrated that no changes occurred in the enamel of the

deciduous teeth of the dogs, but marked changes occurred in the enamel of the permanent teeth. The fluorides were fed to the bitches during lactation, and it is possible that had the feeding occurred during pregnancy the deciduous enamel might have been affected. Since the ingestion of certain fluorides has such detrimental effect upon the enamel of permanent teeth, the question arises whether the fluorides present in water might not affect other tissues or organs or metabolic processes of one kind or another. This problem is receiving study in these laboratories at the present time. It has been found in the case of dogs that oral administration of 0.45 mg. to 4.52 mg. fluorine as sodium fluoride per kilo of body weight caused no effect on total calcium, acid-soluble inorganic phosphorus, hemoglobin, or coagulation time of blood. Radiographs showed no changes in the bones of dogs which received as much as 4.52 mg. of fluorine as sodium fluoride orally per kilo of body weight.

Experiments have been made to determine the effects of organic fluorides on the enamel of the teeth in the hope that there might be some relation between structure of the compound and the capacity of the organism to split off the fluorine atom. Rats were employed as the experimental animal and the following compounds were tested: α-fluoronaphthalene, p-fluorobenzoic acid, p, p^1-difluorodiphenyl, and fluorobenzene. Alpha-fluoronaphthalene was fed at levels of 0.10 and 0.05 per cent of fluorine. Para-fluorobenzoic acid and p, p^1-difluorodiphenyl were administered to the extent of 0.05 per cent of fluorine; whereas, fluorobenzene was given at a level of 0.10 per cent of flourine. The animals receiving 0.10 per cent of fluorine as α-fluoronaphthalene died in twenty days and showed no abnormalities of the teeth. The same compound fed at a level of 0.05 per cent of fluorine produced mottled enamel. The animals on this latter level of α-fluoronaphthalene did not reproduce, although they were more than a year old when the experiment was discontinued. The p-fluorobenzoic acid, p, p^1-difluorodiphenyl, and fluorobenzene did not produce mottled teeth at the levels used. None of the animals on the organic fluorides showed any significant variation in hemoglobin content of the blood.

Experiments were made on the removal of the fluorine from water. In order to study this problem of removal on a large scale, an experimental plant was attached to the mains in one of the schools in the city of Ankeny. This water treatment machine was designed by Professor W. E. Galligan of the Civil Engineering Department and used by him for water purification, softening, and clarification. The plant consists of three mixing tanks, a settling basin, and filters. Sand and gravel, such as are ordinarily used in the filtering of water supplies, were employed for the filter beds. Analyses of fluorine were made by the Boruff and Abbott[9] modification of the Willard and Winter method. Shortly after the discovery of fluorine in the water at Ankeny, experiments were begun by two of the authors (Greenwood and Nelson) to remove fluorine by the addition of aluminum salts, because of the great insolubility of aluminum fluoride. This work was in progress only a short time when Boruff[10] announced that fluorine may be removed from water by treatment with alum (aluminum sulphate). In the experiments to be described, aluminum sulphate has been used to carry down the fluorine from the water.

The data in Table I show that the fluorine content of Ankeny city water has been lowered to an appreciable extent. While addition of HCl to the alkaline Ankeny city water improved somewhat the removal of fluorine in small scale laboratory experiments, such improvement was not so marked in the use of the continuous treatment plant at Ankeny. The addition of small amounts of

TABLE I

THE EFFECT OF ADDITION OF ALUM ON FLUORINE CONTENT OF WATER*

EXPERIMENT	APPEARANCE OF EFFLUENT	C.C. CONC. HCl ADDED PER GAL. WATER	FLUORINE IN EFFLUENT	
			AS RECEIVED	AFTER STANDING
1	Slightly cloudy	0.16	2.73	---
2	Clear	0.16	2.26	---
3	Clear	0.08	2.02	---
4	Very cloudy	None	3.68	1.88
5	Very cloudy	None	3.00	1.50

*The dosage of $Al_2(SO_4)_3.18\ H_2O$ was constant at 20 grains per gallon. The water under treatment contained 8 parts per million of fluorine.

HCl in the continuous treatment caused the flock to settle more rapidly and completely, so that the water from the filter was reasonably clear and, upon standing, gave little sediment. Such was not the case, however, when HCl was omitted, for the flock settled less rapidly and not as completely, resulting in an effluent which was quite cloudy, and which, upon standing, gave much sediment. The cloudy effluents, as received, gave higher values for fluorine than did the clearer ones, but if allowed to stand and then analyzed, sediment free, the originally cloudy effluents gave values lower than the originally clear effluents. We are making more detailed studies of the factors which might influence removal, with the hope of establishing the optimum combination of conditions for practical and more nearly complete removal of fluorine. Laboratory experiments show that alum treatment of solutions of fluorides lowers the fluorine concentration to as much as one part of fluorine per million of solution. When the conditions are known it should, therefore, be possible to remove fluorine from large quantities of water, to a very low level.

Previous work from this laboratory by Keil and Nelson[11] on aluminum in nutrition, indicates that animals can tolerate considerable quantities of aluminum salts. The toxicity of aluminum salts depends on the nature of the salt. Whereas, NaF or CaF_2, when fed to rats, causes mottled enamel, it was observed that the administration of 0.10 per cent of fluorine as Al_2F_6, aluminum fluoride, had no effect upon the teeth. The animals looked well and reproduction was very good on this level of aluminum fluoride intake. It, therefore, seemed possible that, if aluminum sulphate were fed to animals receiving fluorine water, mottled enamel would not develop, and no serious disturbance of metabolism should result. Animals receiving 0.025 per cent of NaF all showed severely mottled teeth. The animals which received 0.025 per cent of NaF plus 0.132 per cent of $Al_2(SO_4)_3.18\ H_2O$ showed some mottling. The male animals receiving 0.025 per cent of NaF plus 0.396 per cent of alum had normal teeth, whereas, the female rats on these levels showed normal teeth until after birth of young, when the mothers exhibited slight mottling. Male animals receiving 0.05 per cent of NaF plus 0.792 per cent of $Al_2(SO_4)_3.18\ H_2O$

showed very slight mottling, whereas, the females, on these levels during lactation, showed somewhat more extensive mottling. All of the alum-fed rats grew normally, and they all reproduced. Second-generation animals on the alum diets are now on experiment. Although Al_2F_6 did not produce mottled enamel, $CaSiF_6$ and CuF_2 were positive in their effect. The experiments show that alum administration is effective in markedly reducing the effect of fluorine, and, no doubt, with larger quantities of aluminum salts, the effect of fluorine upon the teeth can be prevented entirely.

SUMMARY

1. Oral administration of 0.45 mg. to 4.52 mg. of fluorine as sodium fluoride per kilo of body weight caused no effect on total calcium, acid-soluble inorganic phosphorus, hemoglobin, or coagulation time of blood of the dog.

2. Radiographs indicate that there are no changes in the bones of dogs which received 4.52 mg. of fluorine as sodium fluoride orally per kilo of body weight.

3. Alpha-fluoronaphthalene produced mottled enamel; whereas, the ingestion of p, p¹-difluorodiphenyl, p-fluorobenzoic acid, and fluorobenzene had no effect on the teeth.

4. Fluorine in water has been lowered by treatment to 1.5 to 2 parts per million from an original concentration of 8.0 parts per million.

5. Acidity of the water is a factor in the removal of the fluorine by aluminum sulphate. This is in part due to the nature of the floek produced under different hydrogen ion concentrations.

6. Calcium silicofluoride and cupric fluoride caused mottled enamel; whereas, ingestion of Al_2F_6 did not produce this effect.

7. The ingestion of aluminum sulphate, simultaneously along with fluorides, prevents, or at least markedly reduces, the effect of fluorine on the teeth.

REFERENCES

1. Smith, M. C., Lantz, E. M., and Smith, H. V.: Cause of Mottled Enamel, a Defect of Human Teeth, Ariz. Agr. Exper. Sta. Bull. **32**: 253, 1928.
2. Churchill, H. V.: Occurrence of Fluorides in Some Waters of the United States, Ind. & Eng. Chem. **23**: 996, 1931.
3. McKay, F. S.: Mottled Enamel: The Prevention of Its Further Production Through a Change of Water Supply at Oakley, Ida., J. Am. Dent. A. **20**: 1137, 1933.
4. Kehr, R. W.: Dental Deficiencies and Drinking Water, J. Am. Water Works A. **28**: 214, 1931.
5. Ostrem, C. T., Nelson, V. E., Greenwood, D. A., and Wilhelm, H. A.: The Occurrence of Mottled Enamel in Iowa, Science **76**: 575, 1932.
6. Boissevain, C. H.: The Occurrence of Fluorine in the Drinking Water in Colorado, J. Colorado-Wyoming Acad. Sc. **1**: 13, 1933.
7. Dean, H. T.: Distribution of Mottled Enamel in the United States, Pub. Health Report **48**: 703, 1933.
8. Sebrell, W. H., Dean, H. T., Elvove, E., and Breaux, R. P.: Changes in the Teeth of White Rats Given Water From a Mottled Area Compared With Those Produced by Water Containing Sodium Fluoride, Pub. Health Report **48**: 437, 1933.
9. Boruff, C. S., and Abbott, G. B.: Determination of Fluorides in Illinois Waters, Analytical Ed. Ind. and Eng. Chem. **5**: 236, 1933.
10. Boruff, C. S.: Removal of Fluorides From Drinking Waters, Ind. & Eng. Chem. **36**: 69, 1934.
11. Keil, H. H., and Nelson, V. E.: Aluminum in Nutrition, Iowa Acad. Sc. **41**: 161, 1934.

DEXTROSE TOLERANCE IN THE AGED*

MOSES D. DEREN, M.D., NEW YORK, N. Y.

LITTLE attention has been paid to the dextrose tolerance in the aged. Spence[1] was one of the first to show that dextrose tolerance diminishes with advancing years. Blood sugar curves of five men over sixty years of age showed a markedly impaired dextrose tolerance with an average fasting blood sugar of 135 mg. per cent. Hale-White and Payne[2] found in fourteen old people that at the age of seventy the average peak of the blood sugar curve was 220 mg. per cent and at eighty 240 mg. per cent. Punschel,[3] on the other hand, found the average fasting blood sugar in elderly people to be within normal limits. Marshall[4] studied fifty subjects and found that in old people the blood sugar curves fell arbitrarily into five groups: (a) the normal adult, (b) the storage defect, (c) the lag, (d) the flat, and (e) the typical diabetic. The storage type was found to occur most frequently.

EXPERIMENTAL

Two tests for dextrose tolerance have been employed in the present study. The first, designated as the standard test, is the one in general use and consequently has the most data available for comparative study. In this procedure 100 gm. of dextrose are given by mouth after an overnight fast. Blood and urine specimens are collected prior to, and thirty minutes, one hour, and two hours after, the ingestion of the sugar. The blood sugar curves obtained by this method were analyzed according to the classification proposed by Mosenthal.[5]

The second test of dextrose tolerance used was the one-hour two-dose method advanced by Exton and Rose.[6] This procedure is based chiefly on the fact that normal human beings react to repeated doses of dextrose with either hypoglycemia or with little or no change in glycemia, whereas diabetics react with a definite hyperglycemia. One hundred grams of dextrose are divided into two equal parts. Following an overnight fast, blood and urine specimens are obtained. The first dose of dextrose is then given and thirty minutes later a second sample of blood is obtained. The second dose of dextrose is then given and thirty minutes after this blood and urine samples are again obtained. According to Exton and Rose, diminished dextrose tolerance is assumed if the blood sugar in sixty minutes exceeds that of the thirty-minute specimen by more than 10 mg. per 100 c.c. of blood. The fasting blood sugar and the degree of glycosuria are indicative of diabetes with this as in other tests.

*From the Department of Medicine, New York Post-Graduate Medical School and Hospital.
Received for publication, January 2, 1937.
Aided by a grant from the Harriet Weil Fund.

MATERIAL AND METHODS

Fifty subjects over fifty-five years of age and free from acute infections, diabetes mellitus, and glomerular (hemorrhagic) nephritis, were selected from the wards of the hospital. Because of the age group investigated, the patients were admitted to the institution for a variety of conditions, particularly, heart disease, osteoarthritis, hypertension, and arteriosclerosis. These subjects had all been on the same ward diet for at least a week. The fifty subjects were divided into two equal groups. One group was investigated by the standard method and the other group by the Exton-Rose procedure. The blood sugar was determined by the method of Folin and Wu[7] and the urine sugar by Benedict's method.[8]

RESULTS

A summary of the results of the fifty cases studied is given in the protocol; the individual blood sugar curves have been omitted in order to conserve space.

The results with the standard test indicate that in the aged the fasting blood sugar is normal, the highest being 113.4 mg. per cent; the peak of the curve is generally delayed varying from one to two hours; the duration of the curve is prolonged, in most instances exceeding two hours. Sixteen cases with high prolonged curves, five with prolonged and four with normal blood sugar curves were observed. Urine volumes were small and in many instances, none could be voided throughout the length of the test. In the second series studied with the one-hour two-dose dextrose tolerance test, the fasting blood sugar levels are comparable to those of the first group. An analysis of these data according to the criteria postulated by Exton and Rose revealed twenty-one diabetic and four normal types of sugar curves.

COMMENT

Carbohydrate metabolism as measured by blood sugar curves is definitely impaired in old age. Marshall[4] found dextrose tolerance to be diminished more frequently in the old age group with disease than in the normal old age group. In the present series no difference could be found in the blood sugar curves in subjects with heart disease, carcinoma, and arthritis that could not be adduced to old age alone. The blood sugar curves obtained are comparable to those of the twenty-eight healthy subjects of Marshall's series.

TABLE I

SUMMARY OF TWENTY-FIVE BLOOD SUGAR CURVES OF OLD PEOPLE BY THE STANDARD METHOD

	BLOOD SUGAR IN MG. PER 100 C.C.			
		AFTER GLUCOSE		
	FASTING	ONE-HALF HOUR	ONE HOUR	TWO HOURS
Lowest	73.2	118.0	129.2	69.1
Highest	113.4	192.0	227.3	214.3
Average	88.4	146.2	167.5	146.6

TABLE II

SUMMARY OF TWENTY-FIVE BLOOD SUGAR CURVES OF OLD PEOPLE BY THE EXTON-ROSE METHOD

	BLOOD SUGAR IN MG. PER 100 C.C.			
		AFTER GLUCOSE		
	FASTING	ONE-HALF HOUR	ONE HOUR	
Lowest	71.8	91.5	105.6	
Highest	106.3	168.5	223.7	
Average	92.9	132.1	173.3	

It need not be assumed that because a large number of the blood sugar curves in the aged showed a diminished dextrose tolerance, diabetes or a tendency to diabetes is present. Mosenthal[5] has shown that there are many so-called normal persons who have blood sugar curves that indicate impaired dextrose tolerance. The work of Nissen and Spencer[9] is of interest in this regard. They studied the dextrose tolerance in 222 cases of arthritis. Fifty-seven per cent showed diminished tolerance and 43 per cent were normal. Most of the subjects under thirty years of age fell in the normal group while most of the older age group showed impaired dextrose tolerance. Serial tests in a group of thirty-three subjects covering periods from one to nine years showed that even a markedly diminished sugar tolerance does not per se indicate a future diabetic. They also found that the diminished sugar tolerance of the arthritic does not always return to normal at the cessation of activity.

The factors responsible for the diminished dextrose tolerance in the aged are not clear. The answer must be sought for in the functional disturbances of various organs and processes in the body. More detailed knowledge about senescence is needed.

The normal youth, when given an excess of glucose, usually responds with a diuresis. None of the elderly subjects showed a diuretic response following the ingestion of dextrose; in many instances there was even difficulty in obtaining specimens of urine. This confirms the observation made by Bruger and Mirsky[10] that diuresis following glucose ingestion occurred more frequently and was more marked in subjects with normal dextrose tolerance than in those exhibiting a diminished tolerance for sugar.

CONCLUSIONS

1. Dextrose tolerance tests were carried out in 50 subjects over fifty-five years of age. The results indicate that dextrose tolerance in the aged is impaired although the fasting blood sugar is usually normal.

2. The results obtained with the standard test using 100 gm. of dextrose are comparable to those carried out by the procedure of Exton and Rose.

3. Evidence is added to the fact that while every case of diabetes mellitus exhibits a diminished dextrose tolerance, every case showing diminished tolerance to sugar is not one of diabetes.

REFERENCES

1. Spence, J. C.: Some Observations on Sugar Tolerance With Special Reference to Variations Found at Different Ages, Quart. J. Med. **14**: 314, 1920.
2. Hale-White, R., and Payne, W. W.: The Dextrose Tolerance Curve in Health, Quart. J. Med. **19**: 393, 1926.
3. Punschel, A.: Der Blutzucker im hoheren Lebensalter unter besonderer Berucksuchtigung der alimentaren Hyperglykame, Ztschr. f. klin. Med. **96**: 253, 1923.
4. Marshall, F. W.: The Sugar Content of the Blood in Elderly People, Quart. J. Med. **25**: 257, 1931.
5. Mosenthal, H. O.: The Interpretation of Sugar Tolerance Tests. The Common Occurrence of Renal Glycosuria, Med. Clin. N. A. **9**: 549, 1925.
6. Exton, W. G., and Rose, A. R.: The One-Hour Two-Dose Dextrose Tolerance Test, Am. J. Clin. Path. **4**: 381, 1934.
7. Folin, O., and Wu, H.: A System of Blood Analysis. Supplement 1. A Simplified and Improved Method of Determination of Sugar, J. Biol. Chem. **41**: 367, 1920.
8. Benedict, S. R.: The Detection and Estimation of Glucose in the Urine, J. A. M. A. **57**: 1193, 1911.
9. Nissen, H. A., and Spencer, K. A.: Sugar Tolerance in Arthritis, New Eng. J. Med. **210**: 13, 1934.
10. Bruger, M., and Mirsky, I. A.: The Variations of the Urea, Total Non-Protein Nitrogen, and Chloride Concentration in the Blood Following Glucose Ingestion, J. LAB. & CLIN. MED. **19**: 474, 1934.

A STUDY OF IMMUNITY TO STAPHYLOCOCCUS TOXIN IN THE ALBINO RAT*

R. H. RIGDON, M.D., NASHVILLE, TENN.

BRYCE and Burnet[1] have shown that a fair proportion of the domestic rats, mixed black and black and white strains, show staphylococcal antitoxin in their serum. They conclude from their study that the natural antitoxic immunity of rats is acquired as the result of antigenic stimuli from the environment and is not an inborn characteristic. In support of this idea, Bryce and Burnet state that rats possessing natural antitoxin respond to a single injection of toxin with a sharp secondary type response while those lacking antitoxin show no antitoxic response within ten days. Further, with repeated injections of toxin, non-immune rats develop antitoxin and very young rats born of natural immune mothers possess antitoxin which later disappears.

This report deals with the active production of staphylococcus immunity, its rate of development and duration, and a study of passive immunity in the young albino rat.

METHODS AND MATERIALS

The animals used were albino rats of the Wistar strain. Adults were at least 75 days old and weighed 160-275 grams. The young rats were approximately 30 days old when tested for the presence of immunity.

The toxin† was prepared by the method of Parker, Hopkins and Gunther[2] with a few unessential modifications.

*From the Departments of Pathology of the Duke University School of Medicine, Durham, North Carolina, and Vanderbilt University Medical School.

Received for publication, January 7, 1937.

†This toxin was prepared by Dr. A. L. Joyner in the Department of Bacteriology, Duke University School of Medicine.

TABLE II

SUMMARY OF TWENTY-FIVE BLOOD SUGAR CURVES OF OLD PEOPLE BY THE EXTON-ROSE METHOD

	BLOOD SUGAR IN MG. PER 100 C.C.			
		AFTER GLUCOSE		
	FASTING	ONE-HALF HOUR	ONE HOUR	
Lowest	71.8	91.5	105.6	
Highest	106.3	168.5	223.7	
Average	92.9	132.1	173.3	

It need not be assumed that because a large number of the blood sugar curves in the aged showed a diminished dextrose tolerance, diabetes or a tendency to diabetes is present. Mosenthal[5] has shown that there are many so-called normal persons who have blood sugar curves that indicate impaired dextrose tolerance. The work of Nissen and Spencer[9] is of interest in this regard. They studied the dextrose tolerance in 222 cases of arthritis. Fifty-seven per cent showed diminished tolerance and 43 per cent were normal. Most of the subjects under thirty years of age fell in the normal group while most of the older age group showed impaired dextrose tolerance. Serial tests in a group of thirty-three subjects covering periods from one to nine years showed that even a markedly diminished sugar tolerance does not per se indicate a future diabetic. They also found that the diminished sugar tolerance of the arthritic does not always return to normal at the cessation of activity.

The factors responsible for the diminished dextrose tolerance in the aged are not clear. The answer must be sought for in the functional disturbances of various organs and processes in the body. More detailed knowledge about senescence is needed.

The normal youth, when given an excess of glucose, usually responds with a diuresis. None of the elderly subjects showed a diuretic response following the ingestion of dextrose; in many instances there was even difficulty in obtaining specimens of urine. This confirms the observation made by Bruger and Mirsky[10] that diuresis following glucose ingestion occurred more frequently and was more marked in subjects with normal dextrose tolerance than in those exhibiting a diminished tolerance for sugar.

CONCLUSIONS

1. Dextrose tolerance tests were carried out in 50 subjects over fifty-five years of age. The results indicate that dextrose tolerance in the aged is impaired although the fasting blood sugar is usually normal.

2. The results obtained with the standard test using 100 gm. of dextrose are comparable to those carried out by the procedure of Exton and Rose.

3. Evidence is added to the fact that while every case of diabetes mellitus exhibits a diminished dextrose tolerance, every case showing diminished tolerance to sugar is not one of diabetes.

REFERENCES

1. Spence, J. C.: Some Observations on Sugar Tolerance With Special Reference to Variations Found at Different Ages, Quart. J. Med. **14**: 314, 1920.
2. Hale-White, R., and Payne, W. W.: The Dextrose Tolerance Curve in Health, Quart. J. Med. **19**: 393, 1926.
3. Punschel, A.: Der Blutzucker im hoheren Lebensalter unter besonderer Berucksuchtigung der alimentaren Hyperglykame, Ztschr. f. klin. Med. **96**: 253, 1923.
4. Marshall, F. W.: The Sugar Content of the Blood in Elderly People, Quart. J. Med. **25**: 257, 1931.
5. Mosenthal, H. O.: The Interpretation of Sugar Tolerance Tests. The Common Occurrence of Renal Glycosuria, Med. Clin. N. A. **9**: 549, 1925.
6. Exton, W. G., and Rose, A. R.: The One-Hour Two-Dose Dextrose Tolerance Test, Am. J. Clin. Path. **4**: 381, 1934.
7. Folin, O., and Wu, H.: A System of Blood Analysis. Supplement 1. A Simplified and Improved Method of Determination of Sugar, J. Biol. Chem. **41**: 367, 1920.
8. Benedict, S. R.: The Detection and Estimation of Glucose in the Urine, J. A. M. A. **57**: 1193, 1911.
9. Nissen, H. A., and Spencer, K. A.: Sugar Tolerance in Arthritis, New Eng. J. Med. **210**: 13, 1934.
10. Bruger, M., and Mirsky, I. A.: The Variations of the Urea, Total Non-Protein Nitrogen, and Chloride Concentration in the Blood Following Glucose Ingestion, J. LAB. & CLIN. MED. **19**: 474, 1934.

A STUDY OF IMMUNITY TO STAPHYLOCOCCUS TOXIN IN THE ALBINO RAT*

R. H. RIGDON, M.D., NASHVILLE, TENN.

BRYCE and Burnet[1] have shown that a fair proportion of the domestic rats, mixed black and black and white strains, show staphylococcal antitoxin in their serum. They conclude from their study that the natural antitoxic immunity of rats is acquired as the result of antigenic stimuli from the environment and is not an inborn characteristic. In support of this idea, Bryce and Burnet state that rats possessing natural antitoxin respond to a single injection of toxin with a sharp secondary type response while those lacking antitoxin show no antitoxic response within ten days. Further, with repeated injections of toxin, non-immune rats develop antitoxin and very young rats born of natural immune mothers possess antitoxin which later disappears.

This report deals with the active production of staphylococcus immunity, its rate of development and duration, and a study of passive immunity in the young albino rat.

METHODS AND MATERIALS

The animals used were albino rats of the Wistar strain. Adults were at least 75 days old and weighed 160-275 grams. The young rats were approximately 30 days old when tested for the presence of immunity.

The toxin† was prepared by the method of Parker, Hopkins and Gunther[2] with a few unessential modifications.

*From the Departments of Pathology of the Duke University School of Medicine, Durham, North Carolina, and Vanderbilt University Medical School.

Received for publication, January 7, 1937.

†This toxin was prepared by Dr. A. L. Joyner in the Department of Bacteriology, Duke University School of Medicine.

The organism was a hemolytic *Staphylococcus aureus* obtained from an autopsy on a patient whose clinical history was that of agranulocytic angina. The diagnosis was confirmed at the autopsy.

Immunity was produced by intraperitoneal injections of staphylococcus toxin. The presence of immunity in each instance was determined by intraperitoneal injection of a lethal dose of staphylococcus toxin. The survival of an animal following a lethal dose of toxin appears to be a satisfactory criterion for the determination of complete protection. In subsequent work, however, it would be of interest to determine the degree of resistance in terms of staphylococcus antitoxin units.

PRODUCTION OF IMMUNITY TO STAPHYLOCOCCUS TOXIN IN THE ADULT RAT

A total of 25 adult rats were given small injections of staphylococcus toxin for immunization. A lethal dose of toxin was given on the eighth day follow-

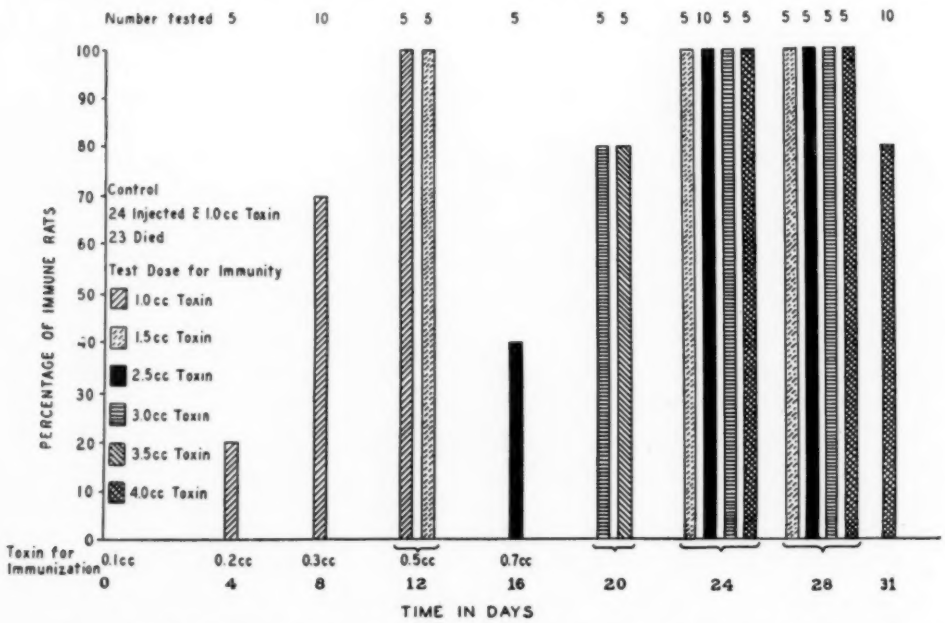


Chart 1.—This graph shows the percentage of adult rats surviving the lethal dose of staphylococcus toxin (1.0 c.c.) at intervals during and after immunization. It also shows the percentage of rats surviving 1.5 c.c., 2.5 c.c., 3.5 c.c., and 4 c.c. of staphylococcus toxin at the different intervals during the experiment. All injections of toxin were given intraperitoneally.

ing the period of immunization. One rat died. A control group of 15 rats was given the lethal dose of toxin and 13 died.

A second group of 25 adult rats was immunized in the same manner. No deaths occurred following the injection of the lethal dose of toxin. There were 27 adult rats in the non-immunized group. After receiving the lethal dose of toxin 26 of these rats died within five hours.

DEVELOPMENT OF IMMUNITY TO STAPHYLOCOCCUS TOXIN IN THE ADULT RAT

The speed of the development of immunity to staphylococcus toxin was determined by immunizing 95 adult rats and determining the number of rats

that survived the lethal dose of toxin at frequent intervals during and after the period of immunization. The results of this experiment are given in Chart 1.

DURATION OF THE IMMUNITY TO STAPHYLOCOCCUS TOXIN DEVELOPED BY THE ADULT RAT

The duration of the immunity to staphylococcus toxin was determined by immunizing a group of 9 rats with the toxin and giving them the lethal dose ninety-nine days later. All the rats survived. Another group of 5 rats immunized the same as the above was given the lethal dose of toxin one hundred thirty-nine days after completing the immunization. One rat died. The control group of 32 rats was given the lethal dose of toxin and 31 died.

PASSIVE IMMUNITY TO STAPHYLOCOCCUS TOXIN IN THE RAT

Female adult rats were given small injections of staphylococcus toxin before, during, and after gestation. Their young were given the lethal dose of toxin at approximately thirty days of age to determine whether or not they were completely protected at this time. In certain cases the mothers were given the lethal dose of toxin shortly after their young were weaned to determine whether or not they were completely immune.

Experiment I.—A group of 5 adult females was given 8 injections of staphylococcus toxin for immunization before they were bred.

Table I gives the number of rats born in each of these litters, the average weight and age and the number surviving the lethal dose of toxin.

Experiment II.—Four adult females were given injections of toxin at the same time as those in Experiment I. An additional injection of 0.5 c.c. of toxin was also given to these rats two to fourteen days before their litters were cast. Table II shows the result of the lethal dose of toxin on the young rats in this group.

TABLE I

This table gives the number of rats in each litter, their age and average weight when the lethal dose of toxin was given, the effects of this dose of toxin, and the length of time between immunization and the time the young were born. The mothers were given 8 injections of toxin before they were bred. In the control group of 28 rats, thirty to forty days of age with an average weight of 73 gm., 24 succumbed following the lethal dose of toxin.

NUMBER OF MOTHER	DAYS FROM IMMUNIZATION UNTIL LITTER CAST	NUMBER IN LITTER	AGE IN DAYS	AVERAGE WEIGHT IN GM.	RESULTS	
					SURVIVED	DIED
6	120	6	28	61	1	5
7	117	6	28	63	1	5
8	120	4	28	55	0	4
9	159	3	28	53	0	3
10	99	6	27	64	1	5
6	152	6	25	37	0	6
7	155	6	27	51	0	6
10	152	6	28	52	0	6

Three more adult females were given 8 injections of toxin before they were bred. A small injection of toxin was also given two to fourteen days before they cast their litters. There were 19 rats in the three litters. Two survived the lethal dose of toxin. In the control group of 13 rats, thirty days of age and averaging 50 gm., 13 died after receiving the lethal dose of toxin.

Experiment III.—Two female rats were given four small injections of toxin before they were bred and four injections during the period of gestation. There were 8 rats in the two litters and only 1 survived the lethal dose of toxin.

Experiment IV.—Two female rats were given one small injection of staphylococcus toxin on the fifth and eleventh day respectively before their litters were cast. The young rats did not survive the lethal dose of toxin. The two mothers also succumbed to the lethal dose of toxin.

Experiment V.—Two females were given three small injections of toxin during the period of lactation. A total of 16 rats were born in the two litters. All died following the lethal dose of toxin. The adult females survived the lethal dose of toxin.

Experiment VI.—One female rat was given a single injection of toxin before casting her litter and four injections during the period of lactation.

The four rats in this litter averaged 63 gm. on the twenty-eighth day. At this time they were given the lethal dose of toxin and all survived. The adult rat was given the lethal dose of toxin at this time and she survived. The control group of 50 young rats averaging 57 gm. was given this lethal dose of toxin and 47 died.

Experiment VII.—Four females were given four injections of toxin at intervals during the period of gestation. The results obtained from the injection of toxin in both the young and their mothers are given in Table III.

TABLE II

This table gives the number of rats in each litter, their age and average weight when the lethal dose of toxin was given, and also the effects of this dose of toxin. The females were given 8 injections of toxin before they were bred and one small injection of toxin two to fourteen days before the litters were cast. In the control group of 28 rats, thirty to forty days of age with an average weight of 73 gm., 24 succumbed following the lethal dose of toxin.

NUMBER OF MOTHER	NUMBER IN LITTER	AGE IN DAYS	AVERAGE WEIGHT IN GM.	RESULTS	
				SURVIVED	DIED
1	6	27	63	5	1
2	5	30	70	5	0
3	6	29	59	0	6
4	6	28	61	5	1

TABLE III

This table gives the number of rats in each litter, their age and average weight when the lethal dose of toxin was given, and also the results of this dose of toxin. The mothers were given 4 injections of toxin during the period of gestation and the lethal dose of toxin after the young were weaned. In the control group of 50 rats, twenty-eight to thirty-two days of age with an average weight of 57 gm., 47 succumbed following the lethal dose of toxin.

NUMBER OF MOTHER	NUMBER IN LITTER	AGE IN DAYS	AVERAGE WEIGHT IN GM.	RESULTS		MOTHERS GIVEN THE LETHAL DOSE OF TOXIN
				SURVIVED	DIED	RESULTS
54	6	29	60	6	0	Survived
55	5	30	62	5	0	Survived
56	3	30	68	3	0	Survived
57	3	29	64	3	0	Survived

DISCUSSION

It is evident from the data that the albino rat is a satisfactory animal to study the immunity produced by staphylococcus toxin. This is shown by the fact that rats developed complete immunity as tested by a lethal dose of toxin given on the twelfth day after immunization was begun. When rats are given five small injections of the toxin at four-day intervals they develop

complete immunity to four lethal doses of toxin by the twenty-fourth day after the first injection. Immunity apparently begins to decline on the thirty-first day after the first injection of toxin as shown in Chart 1.

The duration of complete immunity to the lethal dose of toxin is shown by the survival of rats on the ninety-ninth day following the period of immunization. On the one hundred thirty-ninth day following the course of immunization some of the rats succumb to the lethal dose of toxin. The number of rats is small; however, the data are interesting in that they are indicative of the approximate duration of immunity in the adult rat.

Passive immunity is demonstrated by the fact that every rat born from immune mothers in Experiments 6 and 7 survived the lethal dose of toxin. The mother in Experiment 6 received a single injection of toxin on approximately the fifteenth day of gestation and four injections of toxin during lactation. The mothers in Experiment 7 received only 4 injections of toxin during the period of gestation. All the mothers in Experiments 6 and 7 survived the lethal dose of toxin. From these two experiments it would seem that the antibodies pass through the placenta, colostrum and milk. Needham[3] states that the rat has a hemochorial type of placenta. Kuttner and Ratner[4] state that antibodies do not pass through the colostrum in animals with this type of placenta. In view of this fact it would seem that some rats respond to a single injection of toxin, given during the period of gestation, sufficiently to cause their young to be completely protected against the lethal dose of toxin at thirty days of age. Experiment 4 shows that a majority of rats, however, do not develop a sufficient degree of immunity, from a single injection of toxin, to protect themselves or their young from the lethal dose of toxin.

Experiment 5 supports the observations of others that antibodies do not pass through the colostrum in animals with a hemochorial type of placenta. The two mothers in this experiment survived and each of the 16 young rats died following the injection of the lethal dose of toxin.

It seems that rats immunized to staphylococcus toxin and given a small injection of toxin during the latter half of gestation have a higher percentage of immune animals in their litter than the rats given the same number of injections of toxin and fail to receive an additional injection during gestation. This is shown in Experiments 1 and 2.

From this study on passive immunity in the rat it appears that the antibodies reach the young by passing through the placenta. One injection of toxin during the period of gestation is usually insufficient to produce complete immunity in the young and is also insufficient to protect the mother against the lethal dose of toxin. The greatest degree of protection for the young is obtained by giving several injections of toxin during the period of gestation.

The strength of the toxin used for immunizing the rat is important in studying the speed of development and the degree of immunity at different intervals. If the toxin is weak the degree of protection is less than that found in animals given the same amount of a potent toxin after the same interval of time.

SUMMARY

Rats can be immunized to staphylococcus toxin and appear to be satisfactory animals for such a study. Immunity develops at a relatively rapid pace when a small amount of a strong toxin is given at four-day intervals. Rats so treated were completely protected against four lethal doses of toxin on the twenty-eighth day after injections were begun.

A small group of protected rats survived the lethal dose of toxin ninety-nine days after the last immunizing injection.

The antibodies apparently pass through the rat placenta, and not through the colostrum or milk.

Rats, thirty days of age, born from mothers immunized during gestation or parents recently immunized to staphylococcus toxin and given a single small injection of toxin during the period of gestation, have a sufficient number of antibodies acquired through the placenta to produce complete immunity.

REFERENCES

1. Bryce, L. M., and Burnet, F. M.: Natural Immunity to Staphylococcal Toxin, J. Path. & Bact. **35**: 183, 1932.
2. Parker, Julia T., Hopkins, J. G., and Gunther, A.: Further Studies on the Production of Staphylococcus Toxin, Proc. Soc. Exper. Biol. & Med. **23**: 344, 1925-26.
3. Needham, J.: Placental Barrier. Chemical Embryology, 1931, **3**: Section 21, Cambridge University Press.
4. Kuttner, Ann, and Ratner, Bret: The Importance of Colostrum to the New-Born Infant, Am. J. Dis. Child. **25**: 413, 1923.

GUANIDINE-LIKE SUBSTANCES IN BLOOD*

I. Colorimetric Estimation and Normal Values

Jerome E. Andes, Ph.D., M.D., Morgantown, W. Va., and
Victor C. Myers, Ph.D., D.Sc., Cleveland, Ohio

ALTHOUGH a number of methods for the determination of guanidine-like substances in blood had previously been described, the first procedure in any sense satisfactory was that outlined by Major and Weber[1] in 1927. This method consists briefly of the following steps. The guanidine-like substances in blood are extracted from the Folin-Wu filtrate by means of blood charcoal (in a basic solution), and the guanidines released from the charcoal by treatment with cold acid alcohol. This extract is compared colorimetrically with suitable guanidine standards, using a modification of Tiegs'[2] color reagent to develop the color. Since creatine gives an appreciable color with the reagent, a separate creatine determination is made on the final extract, and a correction subtracted from the final result.

About three years later, Pfiffner and Myers[3] described a modification of this procedure. The most important change introduced was the elimination of the creatine correction, by autoclaving the final extract with HCl (thereby converting the creatine into creatinine). Since our work was completed, Zappacosta[4] has described another method, which is similar to that of Major and Weber, except for the final color development. As a color reagent he uses alpha naphthol and sodium hypochlorite[5] (instead of the ferricyanide-nitroprusside mixture), the reaction being specific for the methyl derivatives of guanidine. Saunders,[6] in 1932, described an entirely different method, which appears to actually separate guanidine and its simple derivatives from other compounds giving the same color reaction. However, this method is of little interest in the study of normal or pathologic blood, as it cannot detect (with any accuracy) guanidine compounds below 2 mg. per 100 c.c. (a concentration rarely reached even in pathologic blood).

EXPERIMENTAL

At the time this work was started, the only satisfactory methods available were those of Major and Weber and of Pfiffner and Myers. Preliminary experimentation soon indicated that the latter procedure was more satisfactory, due to the elimination of the creatine correction. However, several serious objections were found to this method, the more important being as follows: (1) the length of time required, nearly twenty-four hours; (2) the low recovery of added guanidine compounds, about 75 per cent; (3) lack of accuracy in colorimeter readings; and (4) the necessity of preparing the color

*From the Department of Biochemistry, School of Medicine, Western Reserve University. Received for publication, February 3, 1937.

The data presented in this and the following papers were taken from the dissertation submitted by Jerome E. Andes to the Graduate School of Western Reserve University, June, 1932, in partial fulfilment of the requirement for the degree of Doctor of Philosophy.

A preliminary report of the work presented in this and the following papers was given before the American Society of Biological Chemists, Philadelphia, April, 1932, J. Biol. Chem. 97: proc. cix, 1932.

reagent previous to each determination. In attempting to improve upon this method, a number of observations were made, the most important of which will be considered briefly.

In the method of Pfiffner and Myers, the guanidines adsorbed on the blood charcoal are released by allowing the charcoal to stand in contact with acid alcohol overnight. We observed, however, that if the charcoal, containing the adsorbed guanidine, was treated with boiling acid alcohol, better recoveries were obtained than in the overnight treatment with cold alcohol. Furthermore, the liberation of the guanidines seemed to be almost instantaneous. When this modification was introduced into the method of Pfiffner and Myers, the recoveries of methyl guanidine added to blood amounted to about 82 per cent instead of 75. If the final product was not autoclaved, the recoveries amounted to about 89 per cent.

Further studies showed that treating guanidine and its methylated derivatives with boiling acid alcohol did not destroy any of its compounds. Likewise, creatine and creatinine are apparently not affected by the process.

As previously stated, it was observed that the recovery of methyl guanidine added to blood is about 7 per cent greater when the final product is not autoclaved. In fact, the recovery, without the autoclave, is practically the same as the recovery of pure methyl guanidine in an aqueous solution. Since the autoclaving was shown to have no effect on an acid solution of pure methyl guanidine, the 7 per cent loss in the presence of the blood filtrate is probably due to a chemical combination between guanidine and something in the filtrate, in the presence of the hydrochloric acid.

Notwithstanding this loss, the use of the autoclave to convert creatine into creatinine seemed to be far more desirable than to make a separate creatine determination. The use of creatine correction introduces a variable error much greater than the 7 per cent loss produced by the autoclave. Furthermore, the use of the autoclave definitely shortens the time required to complete a determination and reduces the number of manipulations; in addition, it appears to give more consistent and reliable values.

Major and Weber[1] and also Weber[7] have pointed out the fact that blood charcoal converts creatinine into guanidine bases. Tables I and II show the effect of the blood charcoal method on creatinine and creatine added to blood filtrate. The findings indicate that both creatinine and creatine are converted into guanidine by this procedure, and that for the concentrations found in human blood, the conversion is practically proportional to the concentration of creatine or creatinine present. Part of this transformation is produced by the charcoal in basic solution, and part by the process of autoclaving and evaporation to dryness that follows. While the guanidine substances produced from a given weight of creatine are less than with the same amount of creatinine, the amount of guanidines produced per mol of creatine or creatinine is practically the same. Since Major and Weber did not autoclave their product, their values for the conversion of these two substances into guanidines are lower than the results presented in Table I.

Since the amount of guanidine-like substances in the blood is normally very small, in order to make accurate color comparisons the amount of blood

used must be large, or the final volume of the extracted material must be quite small. As a rule not over 50 c.c. of blood filtrate are available, and the guanidine compounds from this quantity of filtrate, contained in 4 c.c. of solution (the smallest volume that can be employed with an ordinary colorimeter), give very little color with the nitroprusside reagent: in fact, the color of the 1 c.c. of color reagent added is much greater than that developed by the guanidine. By fitting a colorimeter with micro cups and plungers, it was found that determinations could be easily carried out with 2 c.c. of material. This departure greatly increases the accuracy of the color comparisons with bloods having a low guanidine content. It was also found that as small an amount as 0.12 gm. of charcoal for the extraction of guanidine bases gave better recovery than larger amounts. Smaller amounts of charcoal were not used due to the difficulty in measuring.

Using the ferricyanide method it was found that guanidine develops about 90 per cent as much color as methyl guanidine. This agrees with Major and Weber's findings,[8] but not with Pfiffner and Myers'.[3] It was also found that creatine gives about one-eighth as much color with the color reagent as methyl guanidine, and that the color given by creatinine (within nine minutes) is negligible. This is in accord with both the findings of Major and Weber and Pfiffner and Myers.

TABLE I

THE CONVERSION OF CREATININE INTO GUANIDINES BY THE GUANIDINE METHOD FOR BLOOD

CREATININE ADDED PER 100 C.C. OF BLOOD	DETERMINED AS METHYL GUANIDINE PER 100 C.C.	METHYL GUANIDINE PER MG. OF CREATININE
mg.	mg.	mg.
30	0.39	0.0133
5	0.07	0.014
30	0.41	0.0137
20	0.31	0.0155
30	0.46	0.0152
Average		0.014

TABLE II

THE CONVERSION OF CREATINE INTO GUANIDINES BY THE GUANIDINE METHOD FOR BLOOD

CREATINE ADDED PER 100 C.C. OF BLOOD	DETERMINED AS METHYL GUANIDINE PER 100 C.C.	METHYL GUANIDINE PER MG. OF CREATINE
mg.	mg.	mg.
20	0.20	0.010
20	0.21	0.0105
30	0.31	0.0103
10	0.09	0.009
20	0.21	0.0105
30	0.30	0.010
20	0.17	0.0085
10	0.09	0.009
Average		0.01

METHOD

Eight cubic centimeters of oxalated blood are pipetted into a 100 c.c. centrifuge tube, and the proteins precipitated by the Folin-Wu procedure, with the exception that the sulphuric acid is added before the sodium tungstate.

The tube is centrifuged and filtered; fifty c.c. of this filtrate are transferred to a 125 c.c. Erlenmeyer flask and rendered basic with 4 or 5 drops of 10 per cent NaOH. To the contents of the flask is added 0.12 to 0.13 gm. (⅛ teaspoon) of blood charcoal (Merck's purified by acid), and the contents shaken well and allowed to stand about two minutes.* The contents of the flask are now filtered through a suction filter, using hard filter paper (7 cm. Whatman No. 2). The flask is rinsed with a few cubic centimeters of water containing NaOH (6 c.c. of 10 per cent NaOH per liter), and the liquid poured through the charcoal on the filter paper. The filter paper and charcoal are removed from the filter, and returned to the original flask; 25 c.c. of alcohol, containing HCl (2 c.c. of 10 normal HCl per liter of 95 per cent ethyl alcohol), are added to the flask immediately.

The flask is placed on a water-bath and the contents evaporated to dryness at a temperature between 80° and 90° C. (higher temperatures cause charring). Evaporation is facilitated by drawing a current of air through the flask. To the dry contents of the flask are added 25 c.c. of 95 per cent alcohol, the contents shaken, and allowed to stand a few minutes. The material is then filtered through a hard filter paper (9 cm. Whatman No. 1 is sufficiently retentive), using an ordinary filtering funnel; 20 c.c. of the resultant filtrate are transferred into a 50 c.c. Erlenmeyer flask, and again evaporated to dryness (the temperature of the bath being kept between 80° and 90° C. as before, and the evaporation facilitated by a current of air).

Two cubic centimeters of 0.2 normal HCl are then added to the flask and autoclaved for fifteen or twenty minutes at about 120° C. to convert any creatine present into creatinine. The flask should be covered with an inverted watch glass during the autoclaving. The contents of the flask are again evaporated to dryness as before, the temperature of the water-bath being not much above 80° during the final stages of the evaporation. Then 1 or 2 c.c. of absolute alcohol are added and the alcohol evaporated (this removes the last traces of HCl present).

Two cubic centimeters of water are now measured into the dry flask containing the extract, and standard solutions are made up containing 0.01, 0.02, 0.03, 0.04, 0.05, 0.07, and 0.10 mg. of methyl guanidine† per 2 c.c. of volume (if ordinary cups and plungers are used, the volumes will have to be doubled). To both the standards and unknown is added 0.5 c.c. of the ferricyanide reagent‡ for each 2 c.c. of volume. The unknown is shaken and poured into a 15 c.c. centrifuge tube and centrifuged for two or three minutes to settle the precipitate that always forms at this juncture. The unknown is com-

*The time factor should be closely adhered to, due to the conversion of creatinine and creatine into guanidine by basic charcoal.

†Methyl guanidine standards are prepared by dissolving 167.1 mg. of methyl guanidine sulphate in 100 c.c. of distilled water. Dilutions of this standard in the ratio of 1:10 and 1:100 are the most convenient for use. The standard keeps perfectly in a refrigerator. Guanidine can be used instead of methyl guanidine, but it develops less color.

‡The reagent is the one described by Major and Weber.[1] It is prepared by mixing the following:

1 part of 10 per cent sodium hydroxide.
1 part of 10 per cent sodium nitroprusside.
1 part of 10 per cent potassium ferricyanide.
9 parts of distilled water (preferably NH_3 free)

The prepared reagent must stand twenty minutes before using. The reagent will keep long periods of time if kept in a refrigerator in brown bottles. A slight precipitate gradually forms at the bottom ($Fe(OH)_3$), but does not appreciably affect the reagent.

pared with the appropriate standard in a colorimeter equipped with micro cups and plungers (a Klett biocolorimeter was used). For most bloods the 0.01 standard is sufficient, and unless nitrogen retention is present, only the 0.01 and 0.02 mg. standard need be prepared. For either the 0.01 and 0.02 mg. standards, a graph had best be employed (the graph is prepared by matching the standard against various concentrations of methyl guanidine on either side of the standard, and plotting the colorimeter readings of these solutions against their concentration). For the standards containing 0.03 mg. or more per 2 c.c. of volume, the guanidine content is proportional to the color, providing the unknown is fairly close to that of the standard.

The reading of the colorimeter should be completed within seven or eight minutes from the time the reagent is added, since the color that the reagent gives with creatinine begins to appear in about nine minutes. The guanidine in the sample is calculated (as methyl guanidine) by the following formula, unless the graph is used.

$$\frac{\text{Reading of Standard}}{\text{Reading of Unknown}} \times \text{Concentration of Standard} \times 25 = \text{mg. of guanidine per 100 c.c. of blood.}$$

The time necessary to complete a determination is not over three hours.

Since creatine and creatinine are partially converted into guanidine compounds by this procedure (or any other procedure using charcoal in a basic solution), a correction must be applied for this conversion. For creatine this correction amounts to about 0.01 mg. of methyl guanidine for each mg. of creatine. For the concentration of creatine found in normal blood, this correction amounts to about 0.03 to 0.04 mg. of methyl guanidine per 100 c.c. of whole blood. The correction is quite constant unless nitrogen retention is present.

For creatinine the correction amounts to 0.014 mg. of methyl guanidine per mg. of creatinine. For the concentration of creatinine found in normal blood this correction amounts to about 0.02 mg. of methyl guanidine per 100 c.c. of blood. This correction is also constant unless nitrogen retention is present.

DISCUSSION OF THE METHOD

It is believed that this method presents certain points of improvement over the previous methods for the following reasons:

1. The use of the Folin-Wu precipitation reagents in the reverse order insures a more rapid precipitation of the blood proteins, the precipitation being quite as complete. The reverse precipitation also gives more filtrate from the same amount of blood. This reverse procedure was also used by Major and Weber.[1]

2. Releasing the guanidine from the charcoal by boiling alcohol has a threefold advantage. It eliminates the guanidine immediately and more completely, and obviates the necessity of allowing it to stand overnight. Second, any charring that might take place on subsequent evaporation, especially the last time, takes place at this time, and the colored material so formed is absorbed by the charcoal. The guanidine compounds are appar-

ently not affected by this process. Finally, this process adds to the accuracy, as a definite amount of liquid can be added to the dry material, giving a known total volume.

3. The use of the suction filter allows rapid filtering and leaves less liquid adhering to the filter paper and charcoal to dilute the acid alcohol added subsequently.

4. The use of a current of air to facilitate evaporation greatly speeds up the method. It is especially important in removing the hydrochloric acid after autoclaving.

5. The use of a microcolorimeter enables the color comparisons to be made with a greater degree of accuracy.

6. The reagent need not be prepared previous to each determination.

7. The use of smaller amounts of charcoal gives a higher recovery of added methyl guanidine, and probably also of the guanidine-like substance in the blood filtrate.

The corrections for creatine and creatinine are practically constant in normal cases, and need not be applied to pathologic bloods except in cases with nitrogen retention. In all the papers of this series the correction for the creatinine conversion was applied, but the correction for the creatine conversion was omitted.

The recovery of methyl guanidine added to blood, as shown in Table III, averages about 82 per cent with this method.

TABLE III

THE RECOVERY OF METHYL GUANIDINE ADDED TO 50 C.C. OF BLOOD FILTRATE

METHYL GUANIDINE ADDED PER 100 C.C. OF BLOOD	METHYL GUANIDINE RECOVERED PER 100 C.C.	PERCENTAGE RECOVERY
mg.	mg.	
2.00	1.65	83
2.00	1.65	83
2.00	1.63	82
2.00	1.65	83
2.00	1.69	85
1.50	1.18	79
1.50	1.17	78
2.00	1.60	80
2.00	1.60	81
2.00	1.66	83
4.00	3.12	78
2.00	1.67	84
2.00	1.68	84
2.00	1.66	83
2.00	1.76	88
2.00	1.61	81
2.00	1.64	82
2.00	1.60	80
1.00	0.78	78
	Average = 82%	

Tables IV and V give the results of blood guanidine determinations on 9 normal males and 17 normal females (during the early part of a normal pregnancy). The males give an average value of 0.25 mg. per 100 c.c. of

whole blood, with a range of 0.21 to 0.28 mg. The average value for females is 0.23 mg. with a range of 0.18 to 0.28 mg. These values are corrected for the conversion of creatinine into guanidines, but not for the conversion of creatine. When the latter correction is also subtracted, the values are about 0.04 mg. lower.

TABLE IV

BLOOD GUANIDINE OF NORMAL MALES

Figures in Mg. per 100 c.c. of Blood

NO.	AGE	BLOOD UREA NITROGEN	BLOOD SUGAR	BLOOD GUANIDINE
1	39	13	83	0.26
2	27	12	95	0.26
3	27	11		0.26
4	28			0.21
5	23			0.28
6	27	11	103	0.25
7	25	13		0.26
8	39	11	108	0.21
9	23	12	110	0.28
Average				0.25

TABLE V

BLOOD GUANIDINE OF FEMALES DURING NORMAL PREGNANCY

NO.	AGE	BLOOD PRESSURE	BLOOD GUANIDINE IN MG. PER 100 C.C.
1	29	90/50	0.28
2	18	124/80	0.24
3	29	98/50	0.26
4	24	90/58	0.24
5	31	100/50	0.28
6	35	110/70	0.23
7	27	120/70	0.25
8	23	94/58	0.26
9	31	110/70	0.18
10	30	105/72	0.19
11	30	110/60	0.22
12	28	90/50	0.18
13	34	102/60	0.27
14	31	132/96	0.27
15	27	140/82	0.28
16	23	105/77	0.23
17	29	120/85	0.23
Average			0.23

These figures agree favorably with those obtained by other methods. Major and Weber give the normal range for their method as about 0.02 to 0.19 mg. per 100 c.c.; other workers report values all the way from 0.00 to 0.50 mg. (the figures usually being below 0.30 if the correction for the color given by creatine is subtracted). Pfiffner and Myers' normal figures are between 0.20 and 0.30; others using the method give values for normal individuals ranging from 0.16 to 0.40 mg.

We would like to add that the identity of the substance (or substances) determined by this or similar methods, is not necessarily guanidine or its simple derivatives. Creatinine and creatine have already been mentioned as being adsorbed and released from charcoal in a manner similar to guanidine, and giving some color with the color reagent. Other compounds, notable

argenine,[9] behave similarly. However, none of these substances (except creatine and creatinine) have been shown to be present in blood in sufficient quantities to exert an appreciable effect on the final color.

SUMMARY

1. A modification of Pfiffner and Myers' method for the colorimetric determination of guanidine bases in the blood is described. The method as modified gives about 82 per cent recovery of added methyl guanidine, and takes less than three hours to complete.

2. Creatine (as well as creatinine) has been shown to be partly converted into guanidine bases by the use of charcoal. Using the method as described, 1 mg. of creatine is converted into about 0.01 mg. of guanidine bases (determined as methyl guanidine), while 1 mg. of creatinine is converted into 0.014 mg. of guanidine bases.

3. The concentration of guanidine-like substances in the blood of 26 normal individuals was determined. For males the average value was 0.25 mg. and for females 0.23 mg. per 100 c.c. of whole blood.

REFERENCES

1. Major, R. H., and Weber, C. J.: Possible Increase of Guanidine in the Blood of Certain Persons With Hypertension, Arch. Int. Med. **40**: 891, 1927.
2. Tiegs, O. W.: A Color Test for Guanidine Bases Together With Some Physiological Applications, Australian J. Exper. Biol. M. Sc. **1**: 93, 1924.
3. Pfiffner, J. J., and Myers, V. C.: On the Colorimetric Estimation of Guanidine Bases in the Blood, J. Biol. Chem. **87**: 345, 1930.
4. Zappacosta, M.: A New Method of Determining Methyl Derivatives of Guanidine in the Blood. Preliminary Observations in Essential and Renal Hypertension, Boll. soc. ital. biol. sper. **10**: 705, 1935.
5. Sakaguchi, S.: Über eine neue Farbenreaktion von Protein und Argenin, J. Biochem. Tokyo **5**: 25, 1925.
6. Saunders, J. A.: A Method for the Isolation of Guanidine, Biochem. J. **26**: 801, 1932.
7. Weber, C. J.: The Determination of Guanidine Bases in the Blood, Proc. Soc. Exper. Biol. & Med. **24**: 712, 1927.
8. Major, R. H., and Weber, C. J.: The Probable Presence of Increased Amounts of Guanidine in Blood of Patients With Arterial Hypertension, Bull. Johns Hopkins Hosp. **40**: 85, 1927.
9. Zappacosta, M.: L'Argenine come causa di aspecificita dei metodi di dosaggio della guanidinemia, Diag. e tec. di lab. **5**: 919, 1934.

SILVER PICRATE TREATMENT OF VAGINAL TRICHOMONIASIS*

LEIB J. GOLUB, B.S., M.D., AND HERMAN A. SHELANSKI, A.B., M.A.,
PHILADELPHIA, PA.

TRICHOMONAS VAGINALIS is believed to be pathogenic in the human vagina and to be the etiologic factor responsible for the symptom-complex of vaginal trichomoniasis, or trichomonas vaginitis. It has been definitely established by many workers that control of this infestation in the vagina produces remission of the characteristic symptoms. Two other pathogenic species have been described in lower animals, *T. columbae*[1] in the pigeon and *T. foetus*[2] in cattle. *T. vaginalis* is the type species of the genus Trichomonas and is one of the three species which may occur in man (Table I). It has been suggested that these species represent three variants of the same organism, produced by differences in environment, but present evidence does not support this view.

TABLE I

CHARACTERISTICS OF THE SPECIES OF TRICHOMONAS FOUND IN MAN

SPECIES	HABITAT	SIZE (MICRONS)	NO. OF FLAGELLA	NUCLEUS	PARABASAL BODY	CHROMATIC GRANULES	UNDULATING MEMBRANE
T. vaginalis	vagina	10 to 36	4	Large, oval, deeply staining	Large, deeply staining	Present	⅓ to ½ length of body
T. hominis	intestinal tract	8 to 16	3, 4, or 5	Small, round, less deeply staining	Not demonstrated as yet	Not demonstrated as yet	Entire length of body
T. buccalis	mouth	6 to 12	4	Small, oval, deeply staining	Small, deeply staining	Present	Two-thirds length of body

METHODS OF DIAGNOSIS

The clinical signs associated with trichomonas vaginitis vary, but certain symptoms are more or less pathognomonic of the infestation. There is present in many cases a profuse, creamy, green gray yellow, thin, bubbly and purulent discharge. In addition to the discharge there is often present an eroded cervix, and a vagina showing a profusion of hemorrhagic spots, giving the "strawberry vagina" picture. Burning and itching of the external genitalia is quite common. Dull pain in the lower left and right quadrants is not unusual. Some patients have all these symptoms, others have one or more, while certain individuals harbor the organisms and clinically present no symptoms. The latter are to be regarded as carriers of the disease and should be treated as thoroughly as those showing clinical manifestations.

*From the Department of Zoology, University of Pennsylvania.
Received for publication, January 20, 1937.

For establishing a definite diagnosis, the patient is placed in the lithotomy position and a sterile, unlubricated speculum is inserted into the vagina. A smear is taken, by means of a sterile wooden applicator, from the regions of the cervix, fornices, external os, and vaginal wall, and the applicator is then placed in a test tube containing three or four c.c. of modified Ringer's solution[3] (NaCl 6.0 gm.; KCl 0.1 gm.; $CaCl_2$ 0.1 gm.; $NaHCO_3$ 0.1 gm.; distilled H_2O 1,000 c.c.). This tube may be kept at room temperature for three or four hours, although it is preferable to make the examination immediately. A few drops of the diluted vaginal specimen are placed on a microslide for examination under the high power dry lens of the microscope. The presence of the organism is readily detected by its motility. This method of diagnosis has proved superior to other procedures and makes possible recognition of varying degrees of infestation. Thus in first grade or mild cases there are few leucocytes, trichomonads, diplococci, or streptococci, while epithelial cells and Döderlein bacilli are numerous. In third grade or severe infestations, this situation is reversed and the field shows few if any epithelial cells or Döderlein bacilli, but there are large numbers of leucocytes, trichomonads, diplococci, and streptococci. Intermediate or second grade cases show varying proportions of the above two main groups of cellular elements. The dark field, hanging drop and glass coverslip methods are not necessary for diagnostic purposes nor is the addition of dyes to stain the surrounding medium in order to make the organisms more distinct. The organism may be seen more distinctly by cutting off some of the light in the microscope.

Staining Method (Giemsa).—The preparation of a permanent slide is not necessary for diagnostic purposes but is necessary where a morphologic study is being made. For this purpose the following method may be used:

1. The material is spread on a microslide or a coverslip by means of a curved forceps, in a thin even film so that it will dry uniformly.
2. Before drying can occur, hold over the mouth of a bottle containing 1 per cent or 2 per cent osmic acid for five to ten seconds.
3. Dry as rapidly as possible, without heat, or the moderate heat of an electric bulb.
4. Fix by immersing in pure methyl alcohol for five to fifteen minutes.
5. Remove and dry in air.
6. Prepare stain by diluting stock Giemsa stain 1:20 with neutralized distilled water, or buffer solution with pH of 6.7.
7. Add dilute stain to smear and leave for thirty to forty-five minutes.
8. Wash in tap water for five seconds.
9. Dry thoroughly without heat.
10. Mount in balsam or examine unmounted with high power dry, or oil immersion lens.

Culture Method.—Inoculate with 1 c.c. of the diluted specimen 10 c.c. of sterile modified Ringer's solution to which has been added, immediately before the inoculation, 0.01 gm. Loeffler's dehydrated blood serum in powdered form. The latter is allowed to settle to the bottom of the tube before the inoculation. Incubate at 37.5° C. for twenty-four hours. Remove a small quantity of the culture from the bottom of the tube by means of a pipette and examine for motile organisms. If present, subculture into a fresh tube of Ringer-Loeffler medium. Subsequent subcultures should be made every forty-eight to seventy-

two hours, depending on the viability of the culture. The addition of 0.01 gm. of powdered gastric mucin to each Ringer-Loeffler tube is advantageous in maintaining the culture over a long period of time.

METHODS OF TREATMENT

In recent years many methods of treatment and many germicidal agents have been used by various workers in the field, with varying degrees of success. A method which has been quite universally employed involves scrubbing the vagina with tincture of green soap, followed by washing with cold water and drying thoroughly. After this procedure the vagina is irrigated with an antiseptic or germicidal solution.[4] However, in cases where the vaginal tract is tender due to an eroded mucosa this scrubbing process is quite irritating. The patient may be relieved during the period of this treatment but it may not be very effective in permanent control of the infestation.

Another type of treatment widely used is the tablet method.[5] In this method too much dependence is placed upon the ability of the patient to insert a tablet as far back as the region of the cervix and fornices. This method does not permit efficient dispersion throughout the entire vaginal tract and the medication may not penetrate all rugae which harbor the organisms.

A preferable form of treatment is the procedure of blowing powder into the vagina while the vaginal tract is distended.[6] In order to accomplish this an instrument must be used which will occlude the entrance to the vagina and permit moderate distention with air by means of a hand bulb. The dispersion of the powder and the distention of the vagina occur simultaneously in the instrument we have used. There is no danger of blowing powder into the fallopian tubes because the pressure can be regulated at the bulb. This enables the medication to penetrate all of the crevices and the rugae and to extend to all parts of the vaginal tract, covering the entire vaginal mucosa and external genitalia.

Among other compounds, picric acid and silver nitrate have both been used in the treatment of *T. vaginalis* vaginitis, but the corrosive action of the latter has been objectionable. Silver picrate has been shown to be more rapidly toxic[7] to *T. vaginalis* in vitro than other silver compounds studied and its use in conjunction with kaolin thus combines a definite toxic action with a useful drying effect.

In the present study, silver picrate* was used in treating 25 cases of *Trichomonas vaginalis* vaginitis. Five grams of a 1 per cent dispersion of silver picrate in kaolin were used, applied to the vagina by means of an occluding insufflator. This was followed by a course of 6 suppositories, each containing 2 gr. of silver picrate. One suppository was inserted by the patient every night, starting the day after insufflation. The patient was instructed to return in one week for another insufflation and 6 more suppositories. Two such treatments were the usual routine. However, more than 2 treatments were given in several cases, as described below. The patients were asked to return after each menstrual period for a re-examination, which was repeated for varying periods of time from about three months to nine months.

*Supplied through the courtesy of John Wyeth & Brother, Inc., Philadelphia.

It is extremely important to emphasize the necessity of removing the plug of mucus, when present, from the external os. This is a precaution against the possibility of the infestation's recurring, because if there are several organisms present in this mucoid plug, they will be dislodged during menstruation and will produce vaginitis once more. For the removal of this mucus, we have employed a colloidal aluminum hydroxide gel containing kaolin, applied on a cotton sponge. This was followed by cleansing of the cervix and vagina with sterile water. This procedure was found to be highly effective, more efficient, and far less irritating than the use of tincture of green soap.

During the course of treatment the patients were instructed to avoid intercourse, douches, and tub baths. Sponge baths are, however, permissible since there is little likelihood of the medication's being removed in that way. No dermatitis, nephritis or any other idiosyncrasies were observed in any of the patients treated.

SUGGESTED METHODS OF TRANSMISSION

The problem of transmission of *T. vaginalis* is one which is as yet unsolved. However, many theories have been advanced by workers in the field. Some believe that, following fecal contamination of the vagina with *T. hominis,* the organism undergoes a change in morphology and becomes *T. vaginalis.* The same theory is advanced in reference to *T. buccalis* when transferred to the environment of the vagina. There is no satisfactory proof that this theory is correct. One of the authors (H. A. S.) has prepared cultures of these three species, in the same medium, and under the same environmental conditions. These cultures were continued for nine months and at the end of that time the organisms were as distinct morphologically as the day they were first incubated. This suggests that the three species will not become a single species, morphologically identical, if placed in the same culture medium and under uniform environmental conditions.

The theory which has been suggested by various workers that the intestinal species, when present in feces contaminating the vagina, will set up a vaginitis just as does *T. vaginalis,* is not acceptable because of the lack of either morphologic or physiologic evidence. However, there appears to be some evidence suggesting a possible reason for a recurrence due to fecal contamination. If feces enter the vagina, the bacteria present could provide an environment favorable to the growth of protozoa. In the event that there are some trichomonads hidden in crevices of the vaginal tract, it is quite possible that they will multiply rapidly in the new favorable medium provided by the fecal bacteria, and cause a flare-up of the vaginitis. On the basis of this theory it would be wise to instruct the patient to cleanse from the vagina to the anus so as to prevent this contamination.

T. vaginalis has been reported several times in prostatic fluid, suggesting that the infestation may be transmitted through intercourse. In our series of cases it was found that in several instances the prostatic fluid of the patient's husband was positive for *T. vaginalis,* confirming the findings of Adair and Hesseltine.[8] Perversion also may play a part in the transmission of the infestation, as well as poor hygienic habits. Cleanliness is undoubtedly a neces-

sary factor in controlling the infestation. In the summer the organisms can survive at room temperature for about twenty-four hours. During this season there is a possibility of transmission of surviving organisms from discharge contaminating plumbing fixtures. In offering these suggestions as to the possible methods of transmission, we would like to point out that little definite information is as yet available.

RESULTS OF TREATMENT

Twenty-five patients with definite infestation by *T. vaginalis* were given the routine treatment outlined above and remained free of the organism after the completion of the indicated number of treatments (Table II). There were four patients in whom symptoms reappeared after the two weeks' treatment, and it was therefore repeated. It was found that in three of these cases (Cases 3, 22, and 23) *T. vaginalis* was present in the prostatic fluid of the respective

TABLE II

DETAILS OF CASES TREATED IN THIS SERIES. DIAGNOSIS AND RESPONSE TO TREATMENT WERE VERIFIED BY MICROSCOPIC EXAMINATION OF FRESH DILUTIONS OF CERVICAL DISCHARGE FOR THE PRESENCE OF TRICHOMONAS VAGINALIS

CASE	AGE	T. VAGINALIS	ASSOCIATED CONDITIONS OR COMPLICATIONS	SYMPTOMS	NO. TREATMENTS	PERIOD OBSERVED AFTER LAST TREATMENT MONTHS	T. VAGINALIS
1	63	+	Prolapse of uterus	Discharge, burning	2	4	-
2	21	+	Cervical polyp (cauterized)	Discharge, itching	3	3	-
3	19	+	Reinfection from husband at end of 3 months	Discharge, itching	2, +1	4	-
4	36	+	None	Discharge, itching, burning	2	6	-
5	32	+	Cervicitis	Discharge and backache	3	4	-
6	48	+	Prolapse of uterus	Discharge and pelvic pains	2	8	-
7	22	+	None	Discharge and itching	2	5	-
8	34	+	Retroversion of uterus	Discharge and abdominal pains	2, +3	6	-
9	41	+	Salpingo-oophorectomy	Discharge and inflammation	4	7	-
10	28	+	None	Discharge and itching	2	4	-
11	50	+	Pruritus	Discharge and itching	2	6	-
12	60	+	Senile vaginitis	Discharge, burning, and pelvic pains	2	5	-
13	20	+	None	Discharge and burning	2	3	-
14	30	+	Cervicitis	Discharge and pelvic pain	3	9	-
15	46	+	Cervical inflammation	Discharge, itching, and burning	2	4	-
16	54	+	Senile vaginitis	Discharge and burning	2	6	-
17	31	+	Retroversion of uterus	Discharge, burning, and itching	2	3	-
18	29	+	*Monilia albicans*	Discharge, itching	3	8	-
19	62	+	Senile vaginitis	Discharge and burning	2	6	-
20	21	+	Cervical inflammation	Discharge and itching	2	4	-
21	32	+	None	Discharge	2	4	-
22	30	+	Reinfection from husband	Discharge and burning	2, +2	4	-
23	52	+	Reinfection from husband	Discharge and itching	2, +1	6	-
24	24	+	None	Discharge and itching	2	3	-
25	28	+	None	Discharge and itching	2	4	-

husbands. In the fourth case (Case 8) symptoms reappeared after the menstrual period following the two weeks' treatment. Three additional treatments were given to this patient and she remained free of symptoms for the six months she was under observation.

SUMMARY

1. A method of diagnosis of *Trichomonas vaginalis* vaginitis is given, including procedures for staining and culturing the organism.

2. Silver picrate, in a 1 per cent dispersion on kaolin, was found to be efficient in rapidly controlling the infestation in twenty-five cases.

3. Methods of transmission are discussed including the presence of *T. vaginalis* in prostatic fluid, which was responsible for recurrence of symptoms in three cases.

The authors wish to express their appreciation to Dr. David H. Wenrich for guidance during the course of this study.

REFERENCES

1. Bos, A.: Über Trichomoniasis bei Tauben, Zentralbl. f. Bakt. **126**: 550, 1932.
2. Abelein, R.: Die Trichomonadenseuche des Rindes und das Scheidenkatarrhproblem, München. tierärztliche Wchnschr. **83**: 318, 1932.
3. Drbohlav, J. J.: Une nouvelle preuve de la possibilité de cultiver Endamoeba dysenteriae, type histolytica, Ann. de parasitol. hum. et comp. **3**: 349, 1925.
4. DeLee, J. B.: Trichomonas Vaginalis Vaginitis, Illinois M. J. **37**: 186, 1920.
5. Karnaky, K. J.: Trichomonas Vaginalis Vaginitis, Urol. & Cutan. Rev. **38**: 174, 1934.
6. Gellhorn, G.: Treatment of Trichomonas Vaginitis With Acetarsone (Stovarsol), J. A. M. A. **100**: 1765, 1933.
7. Shelanski, H. A.: Studies on T. Vaginalis In Vitro, J. Lab. & Clin. Med. **21**: 790, 1936.
8. Adair, F. L., and Hesseltine, H. C.: Histopathology and Treatment of Vaginitis, Am. J. Obst. & Gynec. **32**: 1, 1936.

1829 Pine Street

THE EFFECT ON THE HEART OF EXPERIMENTAL PLEURAL CONGLUTINATION*

HORACE MARSHALL KORNS, M.D., HARRY LANDT, M.D., O. R. HYNDMAN, M.D., RAYMOND GREGORY, M.D., AND C. N. COOPER, M.D., IOWA CITY, IA.

INTRODUCTION

THE idea that extensive conglutination of the visceral and parietal pleurae, particularly in the basal and phrenic regions, interferes with ventilation of the lungs, and thus leads to hypertrophy of the right ventricle, is very old, but from the beginning there was no unanimity on the subject. Ludwig Traube[1] called attention to a passage in Gerhard van Swieten's *Commentarii in Hermanni Boerhaave aphorismos de cognoscendis et curandis morbis* (vol. 2, p. 762, paragraph 843), in which observations recorded in the seventeenth century by Jan Baptista van Helmont, Johann Conrad Peyer, Théophile Bonet, and Isbrand van Diemerbroeck were invoked sometimes for, and sometimes against, the idea. Whereas in Peyer's case the widespread pleural synechiae discovered post-mortem were held responsible for the respiratory embarrassment which had existed during life, "nevertheless it is not to be denied that van Diemerbroeck found the lungs of an executed criminal so firmly adherent not only to the pleura, but also to the entire mediastinum and diaphragm, that they could not be removed without tearing them to pieces. Yet he had enjoyed excellent health, without any respiratory difficulty."† A century later both Morgagni and Joseph Lieutaud adopted Peyer's point of view, and added the suggestion that obliterative pleurisy might give rise to cardiac hypertrophy.

It was not until the latter part of the nineteenth century, after the contributions of James Carson,[2] David Barry,[3] and Frans Cornelius Donders[4] had led to a much clearer understanding of the favorable influence which normal respiration exerts on the movement of blood into and within the thorax, that the possibility of a causal relationship between pleural conglutination and selective right ventricular hypertrophy was considered seriously. As a matter of fact, such a relationship was implicit in Donders' work, but Bäumler[5] and Traube,[1] by correlating ante- and post-mortem observations, made the first deliberate attempt to substantiate it. Their evidence, however, like that of all case reports on the subject, is conflicting and inconclusive. The first of

*From the Department of Internal Medicine, State University of Iowa.

Received for publication, January 25, 1937.

A summary of this work was presented at the eighth annual meeting of the Central Society for Clinical Research, Chicago, Nov. 1, 1935.

†Neque dissimulandum tamen est, quod Diemerbroeckius invenerit in cadavere suspensi furis pulmonem in utroque latere non tantum pleurae sed et toti mediastino et diaphragmati adeo firmiter accretum, ut non sine dilaceratione avelli posset. Interim optime sanus vixerat, absque ulla respirandi difficultate.

Bäumler's three cases was that of a man, aged thirty-four years, who died of cardiac failure. The pronounced hypertrophy and dilatation of both ventricles discovered at necropsy were ascribed, for no better reason than the lack of any other explanation, to the fact that both pleural cavities were completely obliterated. The second and third cases were invalidated by the presence of pathologic conditions other than obliterative pleurisy which might have contributed to the cardiac hypertrophy and dilatation.

Traube did not share Bäumler's opinion that the left ventricle, as well as the right, is affected by pleural conglutination, but his attempt to demonstrate that there is any effect at all was no less unconvincing than Bäumler's. In the report of the only one of his three cases in which an autopsy was performed, there was nothing except pure conjecture to indicate that the con-

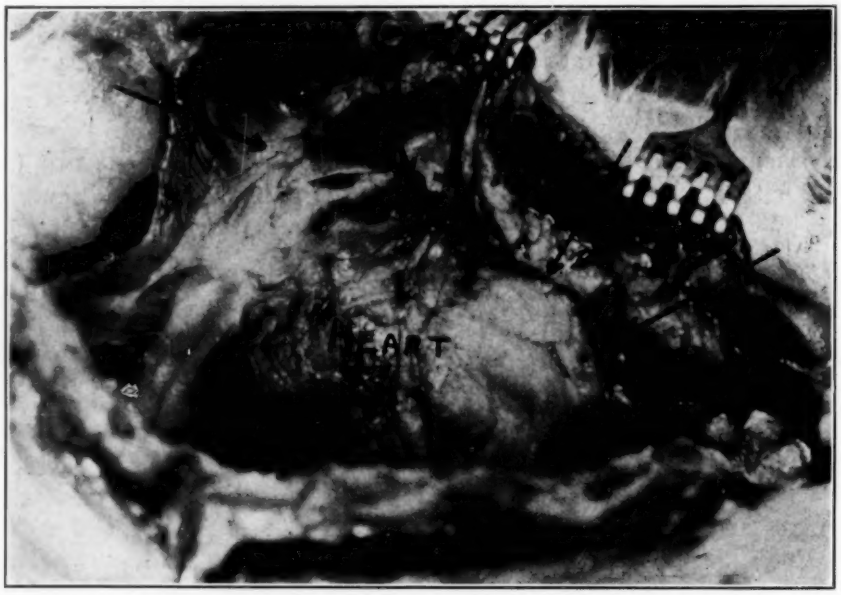


Fig. 1.—Exper. 15B. Photograph taken at necropsy. Complete obliteration of right pleural sac indicated by arrows.

junction of complete bilateral pleural conglutination, bronchial catarrh, bronchiectasis, and hypertrophy and dilatation of the right ventricle was anything but fortuitous. The other cases were dismissed summarily with unconfirmed clinical diagnoses. Thus, in spite of the fact that Traube and Bäumler have been cited authoritatively innumerable times, it is quite obvious that their evidence was purely presumptive.

Hirsch's[6] report, based on five cases, is likewise inconclusive. He did not describe his cases in sufficient detail, or take care to protect his thesis against the possible criticism that there may have been other factors in the right ventricular hypertrophy. His laudable endeavor to verify the hypertrophy by employing Müller's[7] method (now known to be inaccurate) of separating the ventricles for weighing did not fortify his argument in the least. As a matter

of fact, there is no point in studying pathologic material except to learn whether right ventricular hypertrophy invariably accompanies pleural conglutination. If it does, a relationship of some kind may be suspected, but anything resembling proof that the one inevitably entails the other is quite impossible. Only the negative, viz., that they are not directly related, is susceptible of proof by such means.

Theoretically, it is not unreasonable to suppose that some relationship exists. The early work of Carson, Barry, and Donders (loc. cit.) has been confirmed and extended by Hasse,[8] Wenckebach,[9] and Burckhardt,[10] proving beyond question that normal breathing exercises a beneficial effect on blood flow, and Hofbauer[11] and Romberg[12] have argued very plausibly that adhesion of the pleurae impairs this function to so great an extent that the right ventricle must undergo hypertrophy to compensate for the loss. Romberg maintains that the complete obliteration of one pleural sac is sufficient to bring this about.

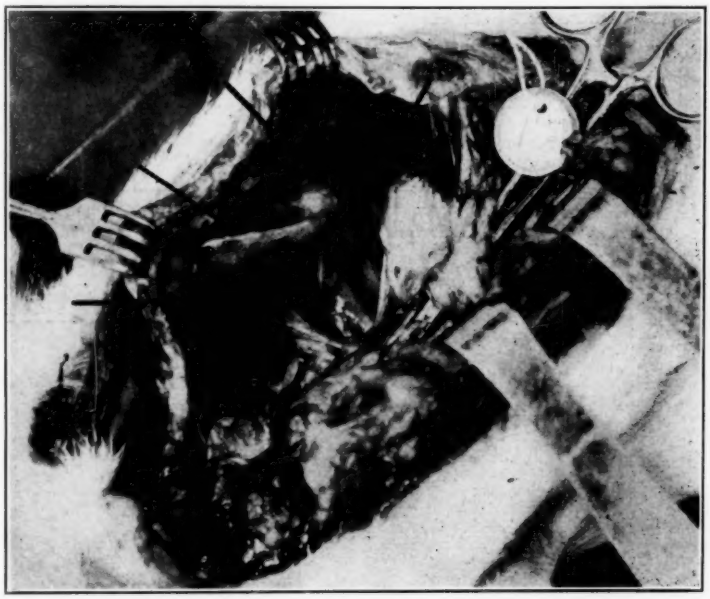


Fig. 2.—Exper. 18B. Photograph taken at necropsy. Arrows point to costal and diaphragmatic adhesions.

As far as we have been able to ascertain, our attempt to elucidate the subject by experimental means is the first that has ever been made.

METHOD

The method of producing pleural conglutination which we employed has been described in detail elsewhere.[13] Briefly, it consisted in placing cotton gauze between the visceral and costal pleura, and over the pleural surface of the diaphragm. Figs. 1 and 2 illustrate, as well as photographs can, the nature of the conglutination which resulted. The hearts were prepared and sectioned by Herrmann's[14] method, the ventricles weighed separately, and the ratio of

TABLE I

EXPER.	BODY WEIGHT (KILOGRAMS)			PLEURAL CONGLUTINATION				L/R RATIO NORMAL: MIN. 1.153 AV. 1.393 MAX. 1.773	REMARKS
	FIRST OPERATION	SECOND OPERATION	POST-MORTEM	LOCATION	EXTENT; IN PER CENT OF TOTAL PLEURAL AREA	DURATION (MONTHS)			
						ONE SIDE	TWO SIDES		
1B			13.8	One-half of the diaphragm only	15	28		1.438	
3B	6.8			Costal only	30	16		1.46	
4B	15.7		13.2	One-half of the diaphragm and small costal area	25	27		1.466	
5B	7.5			Entire diaphragm only	40	15		1.712	
8B	7.1			One entire pleural cavity	50	17½		1.223	
10B			9.3	Costal and phrenic	35	26½		1.4	
13B			9.5	Costal and phrenic	40	24		1.94*	
14B			4.7	Costal and phrenic	30	16		1.301	Empyema; one lung partly collapsed. Animal emaciated
15B			11.3	One entire pleural cavity	50	24		1.44	Very small encapsulated empyema
16B			12.3	Pleuropericardial and phrenic	10	25		1.49	
17B	9.2	12.5	10.7	Right: Costal and phrenic. Left: Costal	65	8	4	1.37	Large empyema and collapse of lung
18B	6.5	7.6	9.0	Costal and phrenic, both sides	25	17	12	1.28	Extrathoracic infection
19B	10.7		14.0	Costal and phrenic, both sides	55	16	12	1.45	Extrathoracic infection
20B	7.5	10.5	13.0	Costal and phrenic, both sides	35	16	12	1.4	
21B	9.5	9.0	12.5	Right: Negligible. Left: Costal and phrenic	25	12½	12	1.7	
22B	10.2	11.5	9.0	Costal only	10	5		1.57	
23B	7.5		7.6	Costal and phrenic	40	5		1.49	Partial atelectasis; small encapsulated empyema
				Average	34				

the left ventricular weight to the right ventricular weight compared with Herrmann's ratios for normal dogs' hearts. Seventeen experiments were completed successfully. The following (19B) is a typical protocol:

Description: Collie, female.

First operation, Jan. 24, 1934:

Weight: 10.7 kg.
Operators: Cooper, Gregory.
Procedure: Five strips of gauze inserted in the left pleural sac in the usual manner Recovery uneventful.

Second operation, May 29, 1934:

Operators: Cooper, Gregory.
Procedure: Five strips of gauze inserted in the right pleural sac, and the right half of the diaphragm covered with gauze. Recovery uneventful.

Necropsy, June 5, 1935:

Weight: 14.0 kg.
Cause of death: Opening the thorax under ether anesthesia.
Right pleural sac: Only the apical area free of adhesion; the remainder, including the entire phrenic surface, completely obliterated. A few pleuropericardial adhesions. Draining sinus externally.
Left pleural sac: One-third of lateral area conglutinated. Phrenicocostal sinus full of adhesions, diaphragm otherwise free.

Ventricular weights (Gregory):

Left ventricle: 48.2 gm.
Right ventricle: 33.7 gm.
L/R ratio: 1.45.

RESULTS

The experimental data are presented in Table I. Most of the animals showed a tendency to gain weight during the period of observation. In five experiments pleuritic adhesions were produced on both sides, and in all but two (3B and 22B) the diaphragmatic pleura participated in the process. The estimated extent of the conglutination varied from 10 to 65 per cent of the total bilateral pleural area, and the duration ranged from five to twenty-eight months. All of the L/R ratios fell within the limits of normal established by Herrmann except the one marked with an asterisk (13B). This is probably an experimental error, but if it were correct it would indicate hypertrophy of the left ventricle, not the right. There is nothing to suggest that the moderately large empyemas which occurred in two animals (14B and 17B) made any difference in the results.

COMMENT

It is obvious that an investigation of this kind should include complete, as well as partial, obliteration of both pleural cavities. It is missing simply because no method capable of producing it was available when the work was being done. If experiments now in progress succeed in supplying the deficiency, a supplementary report will be forthcoming.

In interpreting these experiments, it must not be forgotten that the animal on which they were performed is pronograde. What effect the erect posture might have on the results is purely a matter of conjecture.[15]

CONCLUSION

Partial conglutination of the pleurae, produced experimentally in seventeen dogs, did not lead to selective hypertrophy of the right ventricle.

REFERENCES

1. Traube, L.: Zur Nosologie und Diagnose der totalen Verwachsung beider Pleurablätter, Ges. Beitr. z. Path. u. Physiol. **3**: 338, 1878.
2. Carson, J.: An Inquiry Into the Causes of the Motion of the Blood; With an Appendix, in Which the Process of Respiration and Its Connexion With the Circulation of the Blood Are Attempted to Be Elucidated, Liverpool, 1815, p. 131.
3. Barry, D.: Recherches experimentales sur le cause du mouvement du sang, etc., Paris, 1825.
4. Donders, F. C.: Ztschr. f. rat. Med., Heidelb., n.F. **3**: 39, 1853.
5. Bäumler, C.: Über Obliteration der Pleurasäcke und Verlust der Lungenelasticität als Ursache von Herzhypertrophie, Deutsches Arch. f. klin. Med. **19**: 471, 1877.
6. Hirsch, C.: Über die Beziehungen zwischen dem Herzmuskel und der Körpermuskulatur und über sein Verhalten bei Herzhypertrophie, Deutsches Arch. f. klin. Med. **68**: 321, 1900.
7. Müller, W.: Die Massenverhältnisse des menschlichen Herzens, Hamburg, 1883.
8. Hasse, C.: Die Atmung und der venöse Blutstrom, Arch. f. Anat. u. Entwickelungsgeschichte, 1906, p. 288.
9. Wenckebach, K. F.: Über pathologische Beziehungen zwischen Atmung und Kreislauf beim Menschen, Volkmann's Samml. klin. Vortr., 1907, No. 465/6 (Inn. Med. No. 140/1, p. 131).
10. Burckhardt, H.: Physicalisches über Atmung unter normalen und pathologischen Verhältnissen, Druckdifferenzverfahren und Blutbewegung, Beitr. z. klin. Chir. **110**: 595, 1917-18.
11. Hofbauer, L.: Entstehung und Bekämpfung der konsekutiven Störungen bei Pleuraschwarte, Wien. klin. Wchnschr. **26**: 295, 1913.
12. Romberg, E.: Lehrbuch der Krankheiten des Herzens und der Blutgefässe, Stuttgart, 1925.
13. Landt, H., Hyndman, O. R., and Korns, H. M.: Methods of Producing Experimental Pleural Conglutination, J. Thoracic Surg. **4**: 536, 1935.
14. Herrmann, G. R.: Experimental Heart Disease. I. Methods of Dividing Hearts; With Sectional and Proportional Weights and Ratios for Two Hundred Normal Dogs' Hearts, Am. Heart J. **1**: 213, 1925.
15. Christie, C. D.: Personal suggestion to one of us (H. M. K.).

LABORATORY METHODS

AN ASPIRATOR FOR REMOVING FLUID AND AIR FROM THE PLEURAL CAVITY*†

BURGESS GORDON, M.D., PHILADELPHIA, PA.

VARIOUS methods have been suggested for the removal of air and fluid from the pleural cavity. Their value depends essentially upon the use of suction; needles alone are rarely effective.

The simplest procedure is to employ an ordinary glass syringe, which for the removal of small amounts of fluid is entirely satisfactory. With large quantities the syringe must be detached from the needle repeatedly for emptying, which is tedious, inaccurate and not without danger because of possible contamination. The syringe is also used for the aspiration of air but with limited value. It is difficult to measure the amount of air withdrawn, and there is no means to determine the degree of intrapleural pressure without employing a special needle for connecting with the manometer. A further objection is that air will be sucked back into the pleural cavity while the syringe is being emptied, unless the finger is immediately placed against the needle. As with the aspiration of fluid, the technique is awkward and time consuming.

The Samuel Robinson artificial pneumothorax apparatus is sometimes used for the withdrawal of air. For this purpose the position of the bottles which is ordinarily employed for the introduction of air is reversed. Technically, the procedure is satisfactory, but it may be criticized because of the danger that air will contaminate the apparatus with the possibility that the next patient who receives a pneumothorax treatment will become infected.

A Potain aspirator or one of the modifications is an efficient device for the removal of fluid if a marked negative pressure is maintained in the bottle. An objection is that the bottle and connections are difficult to clean and the latter are not easily kept in good working order. Burrell's bottle is used chiefly in connection with the introduction of air in patients receiving artificial pneumothorax treatments. Gordon's pneumothorax decompressor is intended only for the continuous removal of air in patients with persistently high intrapleural pressures.

The present apparatus has been designed in order to facilitate the removal of fluid and air from the pleural cavity. It consists of a graduated glass syringe enclosed in a metal casing *(D)*; the metal plunger is accurately ground to fit the barrel of the syringe and has a T-shaped handle for convenient manipulation. A metal two-way check valve with a ground-in cone *(B)* opens

*From the Department for Diseases of the Chest, Jefferson Hospital.
Received for publication, January 14, 1936.
†The instrument is manufactured by The George P. Pilling & Son Company, Philadelphia, Pa.

automatically as the plunger is pulled, allowing fluid and air to enter the barrel of the syringe. The instant the outward movement of the syringe is stopped the check-valve becomes closed thus preventing the return of fluid or air to the pleural cavity. The moment this valve closes, another valve of similar design, located in the same housing, automatically opens. With the slightest forward movement of the plunger, air and fluid will be discharged through the valve, the connected nipple *(C)*, and rubber tubing into any convenient receptacle. Two nipples connected with the housing of valve "*A*" are attachments for rubber tubing which lead to the manometer and thoracentesis needle, respectively.

The manometer *(F)*, which is mounted on the same heavy metal base as the syringe, consists of nipple *(H)* for connecting the rubber tubing with valve

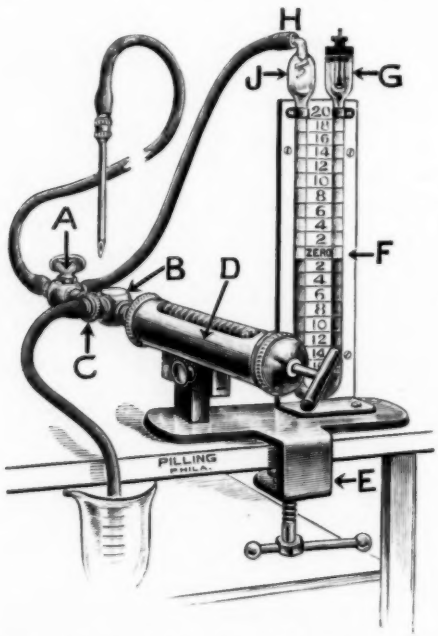


Fig. 1.—Aspirator showing syringe and manometer assembly.

"*A*," a spiral trap *(J)*, and the plunger trap *(G)*, the latter preventing the expulsion of fluid from the manometer. The manometer is of the standard type with unusual visibility of the numerals. The apparatus is fastened to a table by means of a swivel screw.

The apparatus is operated as follows: After the apparatus has been securely fastened to a table, the thoracentesis needle is introduced into the pleural cavity and valve "*A*" is turned to the right. In the presence of a pneumothorax the manometer will show oscillations and indicate the degree of intrapleural pressure; with fluid there will be no oscillations. Valve "*A*" is then turned to the left, and the syringe handle is placed perpendicularly and pulled slowly. With a pleural effusion the fluid will pass into the syringe chamber and the amount may be noted on the graduated scale. After filling,

the handle of the syringe should be pushed forward in order to discharge the contents. This procedure is repeated as many times as necessary, careful count being taken of the amount of fluid withdrawn. In a pneumothorax there is the same manipulation of the syringe but periodically valve "*A*" should be turned in order to determine the degree of intrapleural pressure. The amount of air withdrawn and discharged will be recorded in cubic centimeters as in the case of the fluid.

In cleaning the instrument at least 1,000 c.c. of clear water should be drawn into the syringe and discharged. It is advisable to follow this with 40 c.c. of alcohol and subsequently with 40 c.c. of distilled water.

The advantages of the apparatus are that air and fluid may be conveniently removed and accurately measured; the syringe is fastened to a table and may be manipulated with one hand while the other holds the thoracentesis needle; fluid may be easily drawn through a No. 19 gauge needle in which an ordinary adaptor is used; the intrapleural pressure in pneumothorax cases may be noted at any given time; the apparatus is easily cleaned.

A SIMPLE INEXPENSIVE DEVICE FOR THE REGULATION OF GASEOUS PRESSURES*

AARON EDWIN MARGULIS, M.D., MT. MORRIS, N. Y., AND
GEORGE BLEZINGER, TUCSON, ARIZ.

A SIMPLE, easily constructed and inexpensive device is herein described that will maintain the gaseous pressure in a train of apparatus constant at predeterminable and adjustable levels irrespective of fluctuations (within certain limits) of the source of supply (i.e., pump, aspirator, tank, etc.). Such an apparatus has many applications both in the laboratory and in the clinic. Essentially, it consists of a mercury trap with a mercury reservoir so connected that the height of the mercury column in the valve, and therefore the maximum pressure that can occur in the system, may be varied without interrupting operation. By the transfer of a single rubber tubing connection the apparatus may be used for the regulation of either sub- or supraatmospheric pressures.

The essential features of the instrument are diagramed in Fig. 1. "*A*" is a heavy-walled Pyrex test tube one inch in diameter. The bulb end was cut off and the rim flanged by heating and pressing against a hard surface. A single hole rubber stopper was then forced through the tube from the other end, resulting in a nonejectible closure. A simple two-way stopcock was then inserted into the rubber stopper so that the end of the glass tubing was flush with the upper surface of the stopper. The upper end of "*A*" was fitted with a two-holed rubber stopper. Through one hole was passed a short piece of glass tubing "*S*," which just cleared the lower surface of the cork, while through the other was inserted a piece of glass tubing "*L*," of such length as to extend

*From the Laboratories of the Desert Sanatorium of Southern Arizona.
Received for publication, September 30, 1936.

through "*A*" to within a few millimeters of the upper surface of the lower rubber stopper. The other end of stopcock "*A*" was connectd by rubber tubing to a 100 c.c. syringe "*C*" which serves as the mercury reservoir. Mounted above the valve "*A*" was a three-way glass valve "*D*" of the type that permits any two outlets to be connected together or all three. The central arm is connected by short rubber tubing to a three-way glass connection "E." The side arm of this latter is connected to a manometer "*F*," and the lower by a rubber tubing to either "*S*," or "*L*," as will be explained subsequently. The other two outlets of the three-way valve "*D*" serve to interpose the whole apparatus between the source of sub- or supraatmospheric pressure and the apparatus, the pressure of which is to be controlled. The whole was mounted on a wooden panel with cut out circular areas so that the two lateral arms of the three-way valve "*D*" need not project beyond the board and to facilitate the slipping on of tubing connections. As an added convenience, a suction bottle was mounted behind the upright panel on the base board in a wooden vise (see Fig. 2).

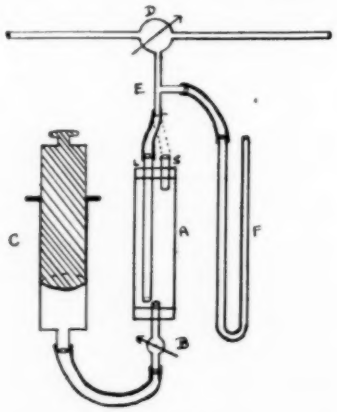


Fig. 1.

To place the apparatus in action, it is simply necessary to fill the reservoir "*C*" about two-thirds full of mercury. This is most conveniently done by removing the upper cork of the tube "*A*," opening valve "*B*" and exerting a slight pull on the syringe "*C*" while pouring mercury into "*A*." If the apparatus is to be used to control subatmospheric pressure, the rubber tubing leading from "*E*" is connected to the short tube "*S*" of the mercury valve "*A*." One of the side arms of "*D*" is connected to the pump with the suction flask interposed, if this be a water pump, and the other arm to the apparatus in which the subatmospheric pressure is to be maintained. The valve "*D*" is then turned so that the arm leading to the pump is connected to "*A*," while the arm connected with the apparatus is blocked off. The pump is then turned on. Mercury is allowed to flow slowly into the valve "*A*" by opening the valve "*S*" until the desired subatmospheric pressure is registered by the manometer "*E*." This will depend, as is apparent, on the height of the mercury column above the lower end of the tube "*L*." The stopcock "*D*" is then turned so that

all three channels are interconnected, i.e., the apparatus to be evacuated is cut in. The water pump can then be turned down so that the air bubbles into the chamber "*A*" in a gentle stream. If it is desired to change the pressure in the system, this can be done without interrupting operation by raising or lowering the level of the mercury in "*A*" while observing the manometer "*F.*" By turning the stopcock "*C*" so that only the two lateral arms are interconnected, the valve can be cut out of the system. If it is desired at any time to raise the pressure in the apparatus, gradually to obviate backflow, this may be done by

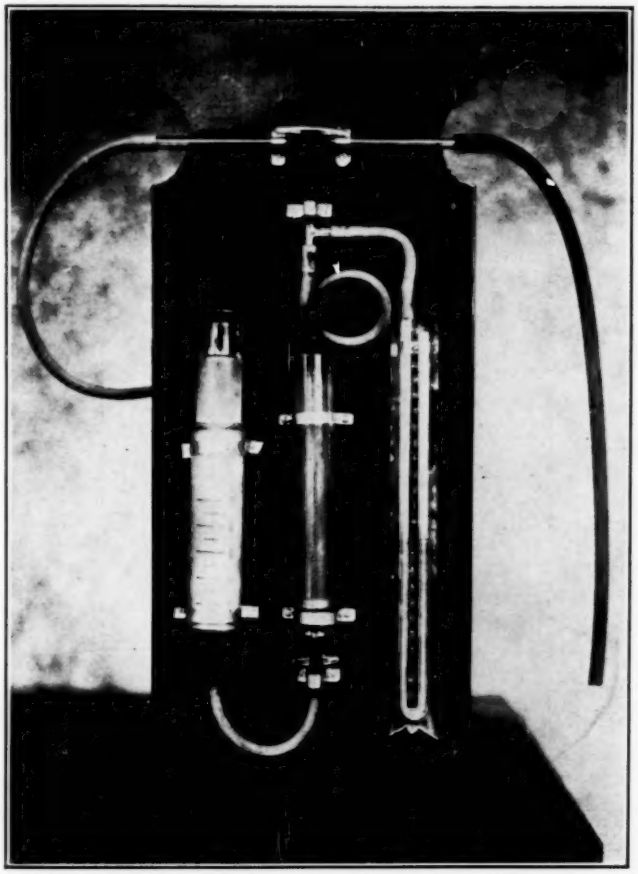


Fig. 2.

turning the valve "*D*" so that the mercury valve is connected only with the side connected to the apparatus, and slowly lowering the level of the mercury in the tube "*A.*"

If the apparatus is to be used to control supraatmospheric pressures, the rubber tubing from "*D*" is connected to "*L*" instead of "*S.*" In all other respects the operation of the instrument is the same. As an added bit of elegance, a four-way glass stopcock, with three side arms and the fourth channel discharging through the cock may be inserted between "*D*" and the mercury valve "*A*" to facilitate this interchange.

The length of the chamber "*A*" should be roughly twice that of the greatest sub- or supraatmospheric pressures expressed in height of mercury column for which the apparatus is to be used. The glass tubes "*S*" and "*L*" should not differ appreciably in bore from the *narrowest* bore interposed between the apparatus and the source of sub- or supraatmospheric pressure.

The above apparatus may be put to many uses. A few may be mentioned: filtration, boiling, or evaporation under controlled, moderately reduced pressure; regulation of aeration in urea determinations; the regulation of gas pressures in tonometers; the continuous evacuation of air or fluids from body cavi-

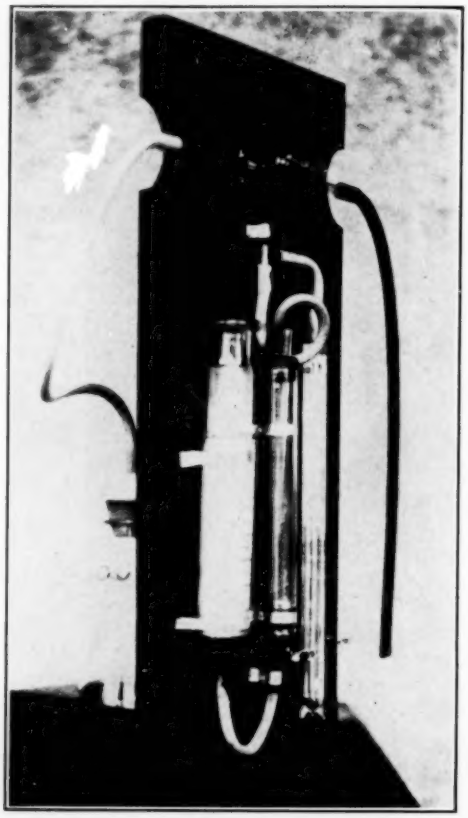


Fig. 3.

ties (e.g., ball valve or leaking spontaneous pneumothoraces, etc.) or from hollow viscı (dilatation of the stomach, inflated bowels) under conditions such that the patient is protected against sudden fluctuations of the pump, or its accidental manipulation; the withdrawal of large quantities of blood from a vein under slight subatmospheric pressures (as in indirect transfusion), et cetera. A similar apparatus has been used in this laboratory for artificial respiration in open chest experiments in dogs. In this instance, a three-way valve was inserted between the trachea of the animal and the apparatus, the degree of pulmonary inflation being easily controlled by variation in the pressure head, and the rate of respiration being controlled by turning the valve by hand, the dog

being allowed to expire through the third arm of the additional three-way valve leading to a tube dipped a few millimeters below a mercury column, which controlled the degree of pulmonary deflection desired. Other uses of the above apparatus will suggest themselves.

SUMMARY

A simple, easily constructed, inexpensive, sub- and supraatmospheric pressure valve employing the principle of the mercury trap is described which is of use in many laboratory and clinical situations.

EXAMINATION OF SUSPECTED SEMEN STAINS FOR SPERMATOZOA*

W. W. Williams, M.D., Springfield, Mass.

OCCASIONALLY in legal medicine it becomes desirable to determine whether stains in articles of clothing, etc., are due to semen. By using suitable methods such may often be determined by the recovery of spermatozoa from the stains.

It happens that spermatozoa once dried on clothing or elsewhere maintain their morphologic characteristics and staining properties over long periods of time, particularly if the articles in question have been kept dry. Then a permanent stained microscope slide preparation of the spermatozoa may readily be prepared from washings of the soiled cloth. The following is the procedure which I have used in detecting spermatozoa: A small piece of the soiled cloth, usually not more than one-half inch diameter, is used. This is placed upon a clean microscope slide, a few drops of saline solution placed upon it, and with the blunt edge of a scalpel the surface of the cloth is scraped off carrying with it any spermatozoa which may be present. The few drops of fluid are spread over the microscope slide. After drying, the film is fixed with heat, and then stained by the following method:

1. Cover film with a 1 per cent solution of Wollschwarz† for five minutes. This solution may be prepared by adding sulphuric acid to a stock 1 per cent solution of Wollschwarz as follows:

Wollschwarz 1 per cent (aqueous)	4.00 c.c.
Sulphuric acid 2 per cent	0.05 c.c.

2. Wash with water.

3. Counterstain six to eight seconds with Loeffler's methylene blue which has been diluted with 15 parts of water. This acts as a mordant to the Wollschwarz, serving to fix it in the nucleus.

4. Wash with distilled water.

5. Dry the film and examine under oil immersion lens.

*Received for publication, November 10, 1936.

†Manufactured by G. Grubler & Co., Leipzig.

This stain imparts to essentially everything aside from the heads of the spermatozoa a dull grayish color, and the heads, standing out in this drab background, will be seen as bright golden or yellowish spots as plain as a beacon

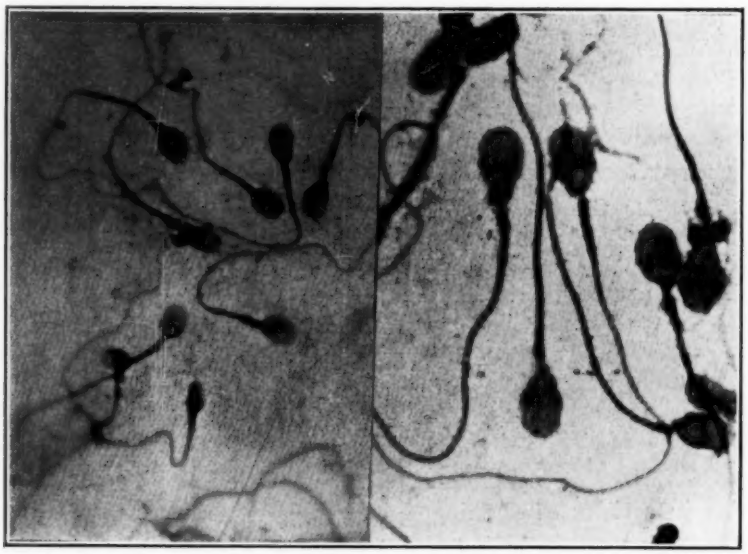


Fig. 1.—Photomicrographs of spermatozoa from normal population. Note the uniformity of size and contour. (Wollschwarz stain.)

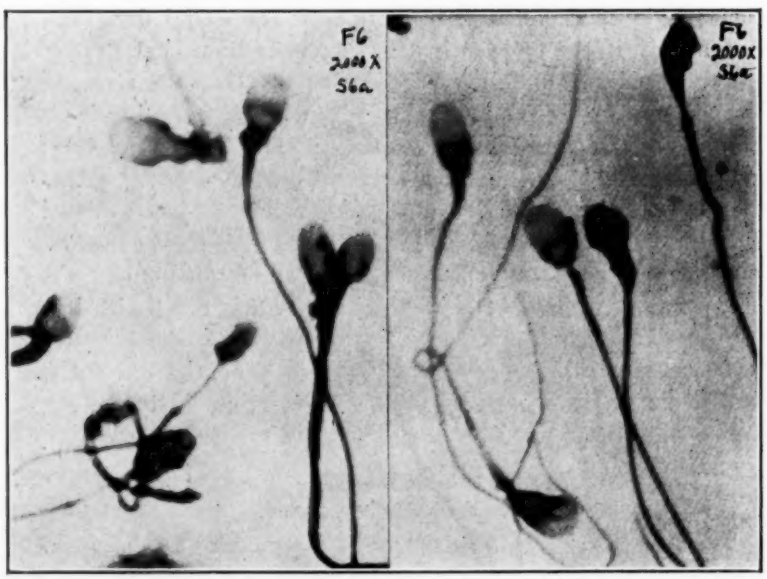


Fig. 2.—Photomicrographs of spermatozoa from pathologic population. Microspermia and various odd-shaped cells abound. (Wollschwarz stain.)

in the night. This stain is exceptionally selective for spermatozoa. With it the nuclei of tissue cells may also be stained, but here the morphology is so different that there needs to be no confusion. Because of the fact that the spermatozoon head maintains its morphologic characteristics rather indefinitely

after drying, this stain serves to readily distinguish spermatozoa by their distinctive morphologic characteristics, and to clearly identify them from any foreign material that may be present.

If plenty of spermatozoa are present, as they often are, the examination need consume no more than five to ten minutes, and one obtains, as a result, a permanent preparation suitable for court evidence.

Another possible use to which such a test might be put is to identify the donor of a given semen sample. Because of the fact that the semen from a given individual presents characteristic spermatic anomalies occurring in constant ratios, it is possible to obtain sufficient information from a properly prepared semen smear to enable one to say positively whether another sample which is submitted for comparison was or was not derived from the same person.

SUMMARY

1. We have given a method for the positive identification of spermatozoa.

2. The microscopic examination not only differentiates spermatozoa of different species, but also may serve to identify different individuals of the same species.

458 Bridge Street

AN IMPROVED STAIN FOR USE IN RAPID BIOPSY DIAGNOSIS*

A. L. Barbrow, M.D., Pittsburgh, Pa.

IN THE rapid diagnosis of biopsies by frozen section, anything which makes for speed and accuracy is worth while. For this purpose we have found the stain herein described eminently satisfactory. It is simple, rapid and sharply differentiating. It is a combination of Van Gieson's stain and Weigert's iron hematoxylin nuclear stain:

A. Hematoxylin	1 gm.
95% alcohol	100 c.c.
This solution should be thoroughly ripened.	
B. Liquor ferri sesquichloratis	4 c.c.
Hydrochloric acid	1 c.c.
Water	95 c.c.
C. 1% aqueous solution acid fuchsin	10 c.c.
Saturated solution picric acid	100 c.c.

The stain is prepared by mixing these three solutions in the following proportions: 5 parts of A, 2 parts of B, and 10 parts of C. It is ready for immediate use. The frozen section is prepared in the usual manner: the slide is flooded with the stain for one minute and quickly rinsed in water. It may then be examined wet or after mounting in balsam. The mixture does not keep well and should be prepared fresh when needed.

2112 Murray Avenue

*From the Laboratories of Homestead Hospital, Homestead, Pa.
Received for publication, October 13, 1936.

A SIMPLE SET OF LABORATORY FORMS*

EDWIN E. OSGOOD, M.D., PORTLAND, ORE.

FOR the benefit of other laboratories, it seems worth while to describe a set of forms which have been in use in the University of Oregon Medical School since 1931. They have been copied by many other institutions and have proved to be labor saving to both the physician and laboratory worker.

UNIVERSITY OF OREGON MEDICAL SCHOOL
OUTPATIENT CLINIC
Sputum

Room No.
Case No. 621
Name N—, R—. H—.
Lab. No. 26559
Date 7/20/36

Diagnosis Bronchial Asthma

Routine	✓	Curschman's spirals +
Hrs.		
Vol.		Vincent's Organisms
Color		Pneumococci
Odor		Streptococci
Pus		T. B. Concentration
Elastic fibres		
Layers	✓	Eosinophils +
B. tuberculosis		Charcot-Leyden cryst.

Ordered by: C. Vetch Examined by: E. Rice Charted by: R. D.

PS16—12098—9-32—2M

Fig. 1.—Color, cream.

UNIVERSITY OF OREGON MEDICAL SCHOOL
OUTPATIENT CLINIC
Stomach Contents

Room No.
Case No. 10776
Name T—, J—.
Lab. No. 116655
Date 7/21/36

Diagnosis Ca of stomach

Meal	Ewald	Total acid	2
Time	1 hr.	Lactic acid	+
Vol.	15 cc.	Occult Blood	+
Appearance	Yellow		
Free HCl	None	Boas-Oppler bacilli	+
Boas HCl	Neg.	Sarcinae	—

Ordered by: H. A. Brown Examined by: E. Rice Charted by: R. D.

PS18—12098—9-32—2M

Fig. 2.—Color, white.

*From the Department of Medicine, University of Oregon Medical School.
Received for publication, July 28, 1936.

They consist of a series of ten forms, one each for hematology, blood chemistry, urine, stomach contents, feces, body fluids, smears and cultures, serology, sputum, and basal metabolic rate. There is also a chart form on which the data from several days' laboratory work may be recorded. The

UNIVERSITY OF OREGON MEDICAL SCHOOL
OUTPATIENT CLINIC
Body Fluids

Room No. 206 Case No. 14076 Name R—, R—.
Lab. No. 116556 Date 7/21/36

Diagnosis Meningitis

	Source	✓	Cell count 151
	Material Spinal fluid	✓	P. M. N. 28
	Vol.	✓	S. L. 72
✓	Appearance Clear		
	Sp. gravity		
✓	Globulin +		
✓	Total Protein		
	Spontaneous coag. 75 mg./100 c.c.		

Ordered by: E. S. Roberts Examined by: Foster Charted by: R. D.

PS12—12098—9-32—2M

Fig. 3.—Color, gray.

UNIVERSITY OF OREGON MEDICAL SCHOOL
OUTPATIENT CLINIC
Urine

Room No. Case No. 32905 Name F—, J—. L.
Lab. No. 116547 Date 7/1/36

Diagnosis Diabetes Mellitus

Routine	✓	New pt.	Microscopic		Special	
✓	Voided		Casts, Hyaline	—	✓	24 hr. vol. 2020
	By catheter		Gran	—	✓	Sugar % 0.96
	Color Yellow				✓	Gm. 24 hr. 19.37
	Appearance Sl. Cloudy					Esbach (gm./L.)
	Reaction Acid		Pus	Occ.		Bilirubin or Biliverdin
	Sp. gravity 1.031		Red cells	—		Bile salts
	Albumin —		Crystals	—		Hemoglobin
	Reduction ++					Indican
	Acetone ++++		Amorphous	Urates +		Urobilinogen
	Diacetic +++		Epithelium	Occ.		
			Bacteria	—		

Ordered by: J. C. E. Examined by: Jaa Charted by: R. D.

PS9—15269—1-36—18M

Fig. 4.—Color, blue.

forms are illustrated in Figs. 1 to 12, Figs. 11 and 12 representing the front and back of the chart form. Each of the forms represented in Figs. 1 to 10 is of a different color so that they can be easily picked out.

These forms (Figs. 1 to 10) serve the purpose of an order form, a label for the specimen, a report form, and a record form. In addition, they have

the advantage that the final record is all in the original writing of the person ordering or doing the work or charting, so that there can be no argument as to who was at fault if any error occurs.

The manner of using these forms is very simple. The physician ordering a test fills in the room number, case number, name of patient, the date, the

UNIVERSITY OF OREGON MEDICAL SCHOOL
OUTPATIENT CLINIC
Basal Metabolic Rate

Room No.
Case No. 7431
Name F—, R—.
Lab. No. 116545
Date 7/1/36

Diagnosis

Age	36	Bar. Pres.	748.5
Height	64 3/4 inches	Temp.	21° C
Weight	208 pounds	Cc. per min.	248
Pulse	56	Rate	−12.2
Temp.	98.6		
Cooperation	Good		

PS24—13187—1-33—2M

Ordered by: H. Round Examined by: See Charted by: R.D.

Fig. 5.—Color, light yellow.

UNIVERSITY OF OREGON MEDICAL SCHOOL
OUTPATIENT CLINIC
Serology

Room No.
Case No. 19041
Name R—, J—. W—.
Lab. No. 26562
Date 7/20/36

Diagnosis Paresis

	Material Spinal fluid		Widal
✓	Kolmer ++++		
✓	Kahn ++++		Blood Group.
✓	Lange 5554320000		Matching

PS27—15269—1-36—12M

Ordered by: G. R. Olsen Examined by: Somes Charted by: R.D.

Fig. 6.—Color, green.

tentative diagnosis, and checks such tests as he wishes to order, checking merely routine, to include all the routine procedures on that type of specimen. He then initials the slip and if he takes the specimen himself, gums the slip to the specimen as a label; otherwise, he sends the slip to the laboratory as an order, and as the specimen is taken the slip is attached as a label. When

the slip is received at the laboratory the name and case number are entered in a book together with a laboratory number, which is stamped on the slip and in the book, and check marks are made in this book under the name of the material and the tests wanted. The statistics of the laboratory are compiled by adding these checks.

UNIVERSITY OF OREGON MEDICAL SCHOOL
OUTPATIENT CLINIC
Hematology

Room No.
Case No. 2104
Name H—, P—.
Lab. No. 26563
Date 7/20/36

Diagnosis Pernicious Anemia

✓ Routine		Special		Special	
Hb %	37.6	✓ Cell Vol.	14.12	Platelets	
Gm.	5.19	✓ Sed. Rate 15 min.	7	Bleed. Time	
R. B. C.	1.14	45 min.	26	Clot. Retr. Time	
W. B. C.	5,150	✓ Reticulo.	6.0%	Coag. Time	
No. Counted	100	✓ Col. I.	1.52	Malaria	
P. M. N.	68	✓ Vol. I.	1.48	Aniso	+
St. Cells	1	✓ Sat. I.	1.03	Poikilo	
P. M. E.	1	✓ Ict. I.	7	Polychrom	+
P. M. B.		Van den Bergh		Macro.	+
S. L.	24	Fragility, begin			
Mon.	6	Fragility, complete			
Disin. cells					

Ordered by: H. R. Lees Examined by: Lee Charted by: R. D.

PS3—15269—1-36—143M

Fig. 7.—Color, pink.

UNIVERSITY OF OREGON MEDICAL SCHOOL
OUTPATIENT CLINIC
Feces

Room No.
Case No. 26301
Name J—, H— P.
Lab. No. 26566
Date 7/20/36

Diagnosis Amebic Dysentery

Routine		Occult blood only	
✓ Color	Brown, blood streaked	Bile pigment	
Odor		✓ Ameba	Endameba histolytica ++
Consistency	Liquid	✓ Flagellates	None found
Occult blood	+++	✓ Worms	None found
Mucus	++	✓ Ova	None found
Pus	—		

Ordered by: Dr. Shaw Examined by: L. Rice Charted by: R. D.

PS7—15387—1-38—1M

Fig. 8.—Color, dark yellow.

When the tests are performed in the laboratory, all calculations are made on the back of the slip and the results are recorded in the space after the test, as illustrated. The technician doing the work initials the slip and the results are then recorded on the patient's chart (Figs. 11 and 12) and the slip is initialed by the person doing the charting, then returned to the laboratory

where these slips are filed in a file according to the institution, a drawer according to the material, alphabetically under the patient's name by current month and current year.

Should a physician state that he ordered a test that is not on the chart, a glance at the slip will show his initials and if there is no check mark by the

UNIVERSITY OF OREGON MEDICAL SCHOOL
OUTPATIENT CLINIC
Blood Chemistry

Room No.
Case No. 60145
Name J—., R—.
26556
Lab. No.
Date 7/20/36

PS21 14182—1-35—2M

Diagnosis Glomerular Nephritis

✓	Urea N. 75 mg./100 cc.		Cholesterol
✓	Creatinine 4.5 mg./100 cc.		Phosphate
✓	Alkali Reserve 29.1		Serum Proteins
	Calcium		
	Dextrose		
			Chlorides
			Uric Acid
			N. P. N.

Ordered by: J. Roberts Examined by: [illegible] Charted by: R. D.

Fig. 9.—Color, rose.

UNIVERSITY OF OREGON MEDICAL SCHOOL
OUTPATIENT CLINIC
Smears and Cultures

Room No.
Case No. 26110
Name H—., S—.
26555
Lab No.
Date 7/20/36

Diagnosis Tonsillitis
Source Throat
Material Pus on swab
G. P. Inoculation

Examine For

	B. Tuberculosis		Pneumococcus
	T. B. Concentration		Typing
	Vincent's Organisms		Gram Neg. Intrac. Dip.
✓	Diphtheria None found		
	Smear:		
✓	Culture: Pure culture — Strep. hemolyticus		

PS30—14182—1-35—4M

Ordered by: M. Carleton Examined by: D. Rose Charted by: R. D.

Fig. 10.—Color, buff.

test, he has nothing further to say. If there is a check mark and a test is not on the slip, the initials of the technician indicate who is responsible. If the result is on the slip but not on the chart, the initials of the person charting indicate responsibility here. A check mark is made in the laboratory book when the slip is returned to the laboratory so that one knows at once whether

UNIVERSITY OF OREGON MEDICAL SCHOOL
PORTLAND, OREGON

OUT-PATIENT CLINIC

LABORATORY RECORD

Name Unit No.

URINE														
Voided														
By catheter														
Vol. 24 hours														
Color														
Appearance														
Reaction														
Sp. G.														
Albumin														
Reduction														
Sugar, per cent														
Gm. 24 hours														
Acetone														
Diacetic acid														
Bile pigment														
Bile salts														
Hemoglobin														
Crystals														
Amorphous														
Casts, hyaline														
Granular														
Pus														
Red cells														
Epithelium														
Bacteria														
SPUTUM, SMEARS, BODY FLUID, ETC.														
Date														
Source														
Material														
Volume														
Color														
Appearance														
Sp. G.														
Globulin														
Esbach														
Cell count														
P. M. N.														
S. L.														
Eosinophils														
Elastic tissue														
Culture														
B. tuberculosis														
G. neg. intrac. dip.														

PM 6—1463—7-35—25M

Fig. 11.—Color, orange. Size 8.5 by 11 inches, margin ⅞ inch.

Blood Hematology										Serology				
Source										Material				
Hemoglobin % (80-140)										Kolmer				
Grams (11-20)										Acetone insol				
R. B. C. (4.0-6.4)										Kahn				
Color index (0.8-1.2)										Lange				
Volume index (0.8-1.2)										Widal				
Sat. index (0.8-1.2)										Type				
Icterus index (4-6)														
Van den Bergh														
W. B. C. (5-10,000)										Stomach				
Number counted										Meal				
Poly. Neut. (55-75%)										Time				
Poly. Eos. (0-4%)										Volume				
Poly. Bas. (0-1%)										Appearance				
S. L. (20-40%)										Free HCl				
Monocytes (3-8%)										Boas HCl				
St. cells										Total acid				
										Lactic acid				
										Occult Bl.				
										B. M. R.				
Normoblasts										Age				
Megaloblasts										Height				
Anisocytosis										Weight				
Poikolocytosis										Pulse				
Polychromataphilia										Bar. Pres.				
Reticulo. (4-30,000)										Temp.				
Sed. rate 15 min. (<5)										Cc. per min.				
45 min. (15)										Rate				
Platelets (250-450,000)										Cooperation				
Coag. Time (< 11 min.)														
Clot retraction														
Bleeding time (<3 min.)														
Feces														
Color														
Odor														
Consistency														
Occult blood														
Mucus														
Pus														
Fat														
Ova														
Parasites														
Blood Chemistry														
Urea N. (7-15 mg.)														
Creatinin (1-2)														
Alkali reserve (50-80)														
Calcium (9-14)														
Dextrose (80-120)														

Fig. 12.—Color, orange.

the slip has been filed. Since the slips are of paper and all in size compared to most record forms, they take up very little room, yet in an experience with over 300,000 tests, they have proved adequate.

The chart form has the great advantage that the physician can tell at a glance whether a laboratory finding is changing by merely glancing across the form instead of thumbing through many pages, as is necessary with many types of laboratory records. The laboratory sheet is of a different color from the other pages of the patient's chart so that one can find it easily.

SUMMARY

A simple set of laboratory forms is described, which have the following advantages: (1) They save writing for the physician, the technician, and the laboratory clerk. (2) One form serves the purpose of four forms, an order form, a label for the specimen, a report form, and a record form. (3) They prevent arguments. They have demonstrated these advantages in actual use in a laboratory where more than 300,000 determinations have been done since their use was inaugurated.

LIPOLYTIC ACTIVITY OF THE LACTOBACILLUS ACIDOPHILUS*

DAVID B. SABINE, B.S., YONKERS, N. Y.

A SIMPLE method for the detection of microbial lipase, described by Berry,[1] can be utilized for the differentiation of strains of the *Lactobacillus acidophilus*. The test consists of streaking the culture on agar to which butter fat has been added. The lipolytic activity is shown by the formation of bluish green insoluble copper soap which appears when the plate, following an appropriate incubation period, is flooded with a saturated solution of copper sulphate. In the following experiments, Torrey's liver glucose agar, as recommended by the author,[2] was used.

Of a collection of 19 strains of *L. acidophilus* isolated from many different sources, 7 failed to show any lipolytic activity. The data are shown in Table I.

With the exception of Strains 3,266 and 3,269, which failed to ferment sucrose and maltose, all the strains fermented dextrose, maltose, sucrose, levulose, lactose, and galactose. The only method of differentiation lay in morphology and colonial form. In several cases these were similar, making classification difficult. Since this situation often arises, it was hoped that the lipolytic reaction might prove of assistance.

A striking example of the value of the test is illustrated by Strains 180 L and 180 S. They were isolated from the same child and their cultural and morphologic characteristics were nearly identical. However, a separation based

*From the Laboratories of the Arlington Chemical Company.
Received for publication, November 19, 1936.

on a slight difference in colony size and a difference in the time of the fermentation reactions was effected. Later, when the lipase test was applied, a clear-cut differentiation was obtained.

TABLE I

NO.	SOURCE	COLONY TYPE	LIPOLYTIC ACT
3264	Commercial concentrate	Y	Positive
3262	Adult's feces	Y	Positive
5	Adult's feces	Y	Positive
B-330	Adult's feces	Y	Positive
3268	Commercial culture	Y	Positive
180 S	Child's feces	Y	Positive
180 L	Child's feces	Y	Negative
A	Commercial concentrate	Y	Positive
M	Child's feces	Y	Positive
3010	Child's feces	Y	Positive
3272 B	Commercial culture	Y	Positive
3266*	Commercial culture	X	Positive
3017 B	Commercial acidophilus milk	X	Negative
3017 A	Commercial acidophilus milk	X	Negative
3270	Commercial acidophilus milk	X	Negative
3269†	Commercial culture	X	Negative
3272 A	Commercial culture	X	Negative
3267	Commercial acidophilus milk	X	Positive
MF	Infant's feces	X	Negative

*Commercial culture of *L. bulgaricus.*
†Labelled acidophilus but its cultural characteristics were more closely allied to bulgaricus.

In clinical work, this test has already proved of value in demonstrating that a strain administered was identical with the strain recovered.

REFERENCES

1. Berry, J. A.: Detection of Microbial Lipase by Copper Soap Formation, J. Bact. **25**: 433, 1933.
2. Sabine, D. B.: A Comparison of Media for Plating L. Acidophilus, J. LAB. & CLIN. MED. **21**: 848, 1936.

INDIVIDUAL CULTURE DISH WITH INCREASED CARBON DIOXIDE TENSION*

AUSTIN L. JOYNER, AND CLAUDIUS P. JONES, DURHAM, N. C

THE use of carbon dioxide to stimulate the growth of certain bacteria has been given particular attention in recent years. McLeod[1] emphasized the importance of carbon dioxide in his description of methods for isolating *Neisseria gonorrhoeae,* and Luther Thompson[2] described a simple method of introducing this gas in measured quantities into culture jars. These methods, in our experience, not only increase the accuracy of diagnosis but very appreciably shorten the time required to demonstrate the organisms.

The purpose of this paper is to call attention to the advantages of the above methods when applied to the Spray anaerobic culture dish. This dish,

*From the Department of Bacteriology and the Department of Obstetrics and Gynecology of Duke University School of Medicine.
Received for publication, November 25, 1936.

described by Spray[3] in 1930, was advocated for use in culturing anaerobes by the alkaline pyrogall method, and we have had excellent results with the technique described by him. The rapidity and ease with which individual cultures could be grown prompted us to use this type of plate to grow cultures under increased carbon dioxide tension.

METHOD

As described by Thompson,[2] "The two solutions to produce carbon dioxide are: (1) a molar solution of sodium bicarbonate (84 gm. to one liter of distilled water); (2) a solution of sulphuric acid (1 c.c. of concentrated acid added to 29 c.c. of distilled water)." The sulphuric acid does not need sterilization but the sodium bicarbonate should be boiled or autoclaved before use. These solutions when mixed in equal parts liberate 22.4 c.c. of carbon dioxide for each cubic centimeter of bicarbonate solution. Since the Spray anaerobic culture dish has a capacity of something over 200 c.c., 1 c.c. of the bicarbonate solution is, therefore, sufficient to create a carbon dioxide concentration of approximately 10 per cent. Solutions are introduced into each side of the partition of the base of the dish with a 1 c.c. pipette. The Petri dish containing the desired medium is placed over the base and sealed with melted paraffin. The assembled dish is then tilted slightly and solutions are well mixed. Care should be taken to allow the paraffin to harden completely before the two solutions are mixed.

DISCUSSION

This combination allows a quick, easy method of preparing individual cultures. The dish is easily inspected without breaking the seal. In our hands enough growth of *Neisseria intracellularis* has been obtained within sixteen hours from spinal fluid in sufficient quantities to obtain test agglutination with specific therapeutic serum. *Neisseria gonorrhoeae* can be isolated in twenty-four hours from vaginal swabs. Specific colonies are picked by means of the McLeod[1] oxydase method.

In one instance cultures of *Neisseria intracellularis* were readily obtained from the blood of a patient showing the clinical symptoms of meningococcus septicemia. Blood cultures by this method should be of particular value when an atmosphere of 10 per cent carbon dioxide is desired.

The extraordinary variety of organisms as well as profusion of growth from cultures of vaginal swabs suggest that this should be a most valuable method of studying this particular group of vaginal flora.

REFERENCES

1. McLeod, J. W., Coates, J. C., Happold, F. C., Priestley, D. P., and Wheatley, B.: Cultivation of the Gonococcus as a Method in the Diagnosis of Gonorrhoea With Special Reference to the Oxydase Reaction and to the Value of Air Reinforced in Its Carbon Dioxide Content, J. Path. & Bact. **39**: 221, 1934.
2. Thompson, Luther: A Simple Method of Supplying Carbon Dioxide in Jars for Bacteriologic Cultures, Am. J. Clin. Path. **5**: 313, 1935.
3. Spray, Robert Spalding: An Improved Anaerobic Culture Dish, J. Lab. & Clin. Med. **16**: 203, 1930.

A SIMPLE CENTRIFUGATION METHOD FOR THE DIAGNOSIS OF SYPHILIS*

F. RYTZ, MINNEAPOLIS, MINN.

IN THE present centrifugation method for the diagnosis of syphilis are embodied the same principles for blood as earlier described for spinal fluid.[1] This method requires very little equipment, a minimum of technical skill, and only a few brief moments for its performance. It is based on the importance of the specific gravity in relation to the relative surface tension of the serum-antigen mixture. The depth of the serum-ammonium sulphate-antigen mixture in the test tube must approximate the inside diameter of the tube. If the depth of the serum-sulphate-antigen mixture in the test tube used is greatly out of proportion to the diameter of the surface, the latter being relatively decreased, the combined antigen-antibody will not, during the process of centrifugation, be forced to the surface as desired, and the combination is then too infirm for further manipulation of the flocculate. The test would at the same time be less sensitive. For that reason the diameter of the test tube must be in proportion to the amount of serum used, and that amount in turn dictates the quantity of antigen emulsion, which is equal to the amount of half saturated ammonium sulphate: 1 part of the emulsion to 3 parts of the serum. By that combination the specific gravity is at the same time brought to a desirable point, and this technique eliminates entirely the heating of the serum or the blood.

Both citrated whole blood and unheated serum are satisfactory for the test. In cases of emergency, it is most convenient to use freshly citrated, unheated whole blood, but the test must then be performed within two hours after collecting the sample. If the test cannot be performed within that time, unheated serum is preferable. For this method, if serum is used, it is important to avoid hemolysis.

PREPARATION OF THE ANTIGEN

Fifty grams of Difco beef-heart powder are placed in 300 c.c. of anesthesia ether, contained in a 1 liter Erlenmeyer flask, and shaken for five seconds every ten minutes for one hour. The suspension is then filtered through a dense filter paper and the powder allowed to dry completely by spreading it out on the filter paper. The dry powder is again placed in 1 liter flask to which is added 300 c.c. of pure acetone. This is shaken for five minutes and then filtered as before. To free the powder from the last drops of acetone, it is pressed lightly with a tongue blade covered with tin foil. In order to dry completely, the powder is placed in the incubator for three to six hours. The dried powder is then weighed and suspended in absolute ethyl alcohol in the

*From the Clinical Laboratories of the Minneapolis General Hospital.
Received for publication, December 7, 1936.

proportion of 1 gram of powder to 5 c.c. of alcohol. This suspension is shaken by hand or in a mechanical shaking apparatus for one and one-half hours and then filtered immediately through a dense filter paper. The alcoholic extract is measured, and 0.6 per cent cholesterol is added. This is dissolved by shaking in a glass-stoppered bottle, or by rotating the bottle in a water-bath at about 50° C. for ten minutes. The cholesterolized extract is filtered. The antigen is then ready for use. It is stored in a brown, glass-stoppered bottle at room temperature and protected against light. This antigen will remain stable for many months.

EMULSIFICATION AND DILUTION OF THE ANTIGEN

Into a large test tube is measured 0.6 c.c. of 0.45 per cent sodium chloride solution. To that is added slowly 0.7 c.c. of 1 per cent cholesterol in absolute alcohol. This is rotated lightly for a few seconds. Then 0.6 c.c. of antigen is added slowly, and the mixture is shaken vigorously for about one minute. The emulsion is diluted with 2.5 c.c. of 0.9 per cent saline, and the tube is again shaken vigorously for one minute. The antigen emulsion is then ready for use. For this method, special titration is not required, and the emulsion will remain stable for about forty-eight hours at a temperature of 20° C.

PERFORMANCE OF THE TEST

The size of the tube should be approximately 9 mm. (inside diameter) by 75 mm. The unit of serum is 0.15 c.c. If citrated whole blood is used, the unit is 0.2 c.c.

1. The unit of unheated serum or freshly citrated whole blood is measured into the respective tubes.

2. To each tube is added 0.05 c.c. of half saturated ammonium sulphate. This is mixed by shaking the rack for a few seconds.

3. Into each tube is then measured 0.05 c.c. of antigen emulsion, and the rack is again shaken to mix the contents.

4. The tubes are centrifuged at the rate of about 2,000 revolutions per minute for three minutes. If that velocity cannot be obtained, the time is extended accordingly.

5. The tubes are removed from the centrifuge and to each is added 3 c.c. of distilled water. By placing the index finger over the mouth of the tube, it is inverted twice without shaking the contents. This is important. If whole blood is used for the test, the fluids are, without inverting the tubes, poured into other tubes with identical numbers, leaving the blood cells at the bottom of the first tube, which can then be discarded.

6. All tubes, with the flocculate or the emulsion suspended in 3 c.c. of distilled water, are again centrifuged at the rate of about 2,000 revolutions per minute for two to three minutes, thus collecting the sediment at the bottom of the tubes.

7. After the tubes have been removed from the centrifuge, the fluid content is decanted by completely inverting a handful of tubes, allowing drainage to the last drop. The inverted tubes are shaken once to insure complete drainage.

8. To the sediment of each tube is added 1 c.c. of 0.9 per cent saline. Before reading the results, each tube is inverted once by placing the index finger over the mouth of the tube.

9. The results are easily estimated against a favorable light without the aid of special facilities. In negative results, the emulsion scatters readily by inverting the tube. A positive test has large floccules in a crystal clear fluid. A weakly positive reaction consists of smaller floccules in a slightly hazy fluid. Weak reactions should be repeated on the same sample, and, as with any test, checked by one or two other methods of known relative value.[2] The test previously described by the authors[3] may conveniently be used for such checking, as highly decomposed blood is satisfactory for that method, and the antigen for the present test is satisfactory for both methods.

SPINAL FLUID

Except for the difference in antigen, which permits omission of the three-minute shaking, the technique for spinal fluid is identical with the test previously reported.[1] The fluid should not be over three days old. Traces of blood render the spinal fluid unsatisfactory for the test. A cloudy fluid should be centrifuged before performance of the test. For the spinal fluid test, the emulsion should be prepared not more than one hour before use.

1. Of unheated spinal fluid 0.2 c.c. is measured into the respective tubes of the same size as described for the blood test.

2. To each tube is added 0.1 c.c. of half saturated ammonium sulphate. This is mixed by shaking the rack vigorously for a few seconds.

3. Then 0.1 c.c. of half saturated sodium chloride is added, and again the rack is shaken to mix the contents.

4. To each tube is measured 0.05 c.c. of antigen emulsion. This is mixed by shaking the rack.

5. The tubes are then centrifuged at the rate of about 1,500 revolutions per minute for fifteen minutes. If that speed cannot be obtained, the time is extended accordingly.

6. After the tubes have been removed from the centrifuge, 3 c.c. of 0.45 per cent saline are added to each tube, and all tubes, one by one, are inverted 10 times by placing the index finger over the mouth of the tube.

7. The tubes are again centrifuged, at the rate of about 2,000 revolutions per minute, for three minutes, collecting the floccules or the emulsion at the bottom of the tubes.

8. The fluid content is decanted in the manner described for the blood test, allowing complete drainage of the tubes by careful manipulation to retain the sediment at the bottom of each tube.

9. To each tube is added 1 c.c. of 0.9 per cent saline. Before reading, each tube is inverted slowly once or twice without shaking the contents.

10. The results are estimated against a favorable light. The floccules from spinal fluid are somewhat finer than those resulting from the blood test. A strongly positive reaction shows fairly large floccules in a clear fluid. A weakly positive reaction has smaller floccules in a slightly hazy fluid. Nega-

tive tests consist of unflocculated emulsion. Even weak positive reactions are important as false positive tests are very rare. A special lamp for reading the results may be of value to the inexperienced operator.

RESULTS OBTAINED

By this method 5,000 blood tests and over 800 spinal fluid reactions have been compared with several other methods of known relative value.[2] Among the blood samples were over 500 from patients in various stages of syphilitic infection. In cases of treated syphilis, this method proved of equal value to the test previously reported,[3] which is one of the most sensitive reactions in treated cases of syphilis. In congenital and primary syphilis, this centrifugation method proved to be more sensitive.

On spinal fluid this method proved to be more sensitive and more reliable than the Wassermann reaction as performed in our laboratory.

SUMMARY

1. A simple and reliable centrifugation method for the diagnosis of syphilis has been described.

2. This method appears to be as sensitive and as specific as any syphilis test used in this country.

3. For this centrifugation method unheated serum and citrated whole blood are satisfactory.

4. This test can be performed with very little equipment as the water-bath, shaking apparatus, special lamps and hand lenses are not required.

5. The antigen is simple to prepare and will remain stable for many months.

6. The test requires but a few minutes for its performance.

REFERENCES

1. Rytz, F.: A Rapid Flocculation Method for the Diagnosis of Syphilis: Technic for Spinal Fluid, J. Lab. & Clin. Med. **22**: 82, 1936.
2. Cumming, H. S., et al.: The Evaluation of Serodiagnostic Tests for Syphilis in the United States, J. A. M. A. **104**: 2083, 1935.
3. Rytz, F.: A Rapid Flocculation Method for the Diagnosis of Syphilis, J. Lab. & Clin. Med. **21**: 934, 1936.

A FILTER FLASK FOR DISPENSING FILTRATES ASEPTICALLY*

THOMAS C. GRUBB, PH.D., SPRINGFIELD, ILL.

THE usual method of dispensing filtrates after they have passed through a Seitz or Berkefeld filter into the filter flask is by pipetting or pouring the filtrate out of the flask. It is obvious why filtrates dispensed in this manner frequently become contaminated with air-borne microorganisms during the

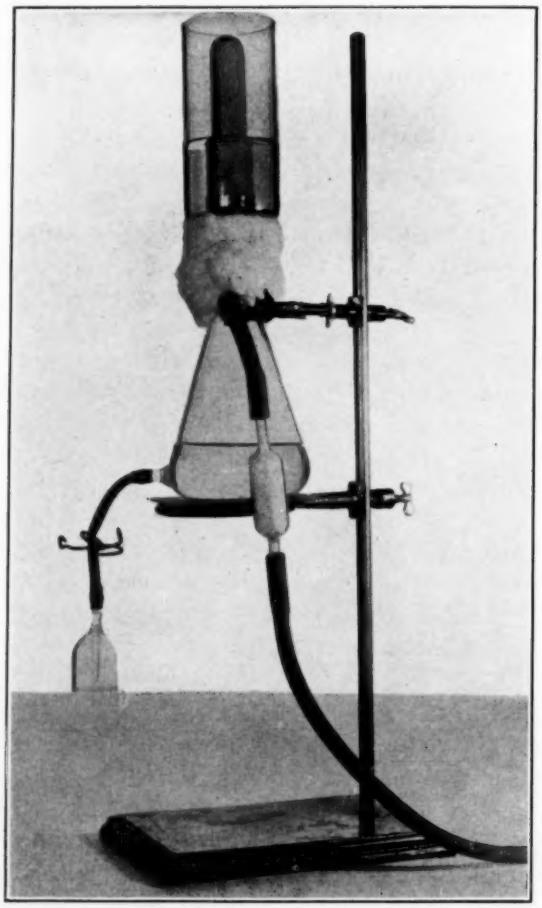


Fig. 1.

process. Those who have worked with or who are familiar with the literature on filtrable forms of bacteria are too well acquainted with air contaminants as a possible source of growth in filtrates and the consequent questionable nature of any "filtrable forms" obtained.

*From the Division of Laboratories, Illinois State Department of Public Health.
Received for publication, December 14, 1936.

In order to obviate the possibility of air contamination while dispensing sterile filtrates, a flask has been designed which combines the essential features of an aspirator bottle and an ordinary filter flask. Fig. 1 indicates how the flask is employed with a Berkefeld candle and a glass bell filling device for filtering and dispensing culture media, sugar solutions, etc.* This apparatus practically precludes any possibility of air contamination in dispensing sterile filtrates.

To prepare the flask for use, either a Berkefeld or Seitz filter is fastened in the top of the flask with a single hole rubber stopper. A strip of cotton is fastened around the joint between the rubber stopper and the lip of the flask to prevent contamination should the stopper become loose in handling. The side tubulation may be plugged with cotton or connected to a glass bulb stuffed with cotton as shown in the figure. A glass bell filling device, completely covered with tin foil or heavy paper, is connected to the lower tubulation with a short piece of thin-walled rubber tubing. The entire apparatus is then placed in the autoclave for sterilization.

After the apparatus has cooled, it is placed on a ring stand as shown in the figure. The side tubulation is connected with the source of vacuum and a pinch clamp is fastened on the rubber tubing just below the lower tubulation. The material to be filtered is poured into the filter and the vacuum turned on. When a sufficient amount has been filtered, the vacuum is turned off, the covering removed from the bell filling device and test tubes, flasks, etc., are filled by inserting them inside of the filling device and releasing the pinch clamp. The usual precautions of flaming the tubes before and after filling should be observed with an occasional flaming of the mouth of the filling device.

This apparatus finds many uses in the routine and research bacteriologic laboratory where culture media, toxin broths, serum, sugar solutions, etc., must be sterilized by filtration and aseptically dispensed. In our laboratory this apparatus is chiefly used for filtering large quantities of serum used in the preparation of various types of media.

*The flask and filling device may be obtained in various sizes from the Corning Glass Works, Corning, N. Y.

DETERMINATION OF THE ICTERIC INDEX BY THE ACETONE METHOD*

ROBERT A. NEWBURGER, M.D., NEW YORK, N. Y.

IN THIS report an attempt will be made to show that, by using acetone rather than water or saline as a diluent, the determination of blood icteric index is rendered more accurate. The clinical course of jaundiced patients may be followed more precisely by this method, which may, therefore, frequently serve as a useful alternative procedure to the more complicated quantitative van den Bergh reaction. The latter, moreover, possesses the additional disadvantage of unreliability when the amount of circulating bilirubin is less than 0.5 mg. per cent.[1]

INTRODUCTION

To meet the demand for a simpler and more satisfactory quantitative method than that of van den Bergh,[2] Meulengracht[3] attempted to measure bilirubin colorimetrically by comparison of the serum with a standard solution (1:10,000) of potassium dichromate. He diluted the serum with normal salt solution, and used, as an arbitrary unit, the number of times the serum had to be diluted to match the standard, the resulting value being designated the icteric index. Meulengracht was of the opinion that bilirubin is in almost all instances the only colored substance to be found in the serum in sufficient concentration to affect the readings significantly.

In 1924, Ernst and Förster[1] reported difficulties arising in the use of the Meulengracht technique. They found that diluting with saline frequently rendered the serum opalescent, causing marked difficulties in matching with the clear bichromate standard. In addition, hemolysis, when present, made accurate comparison impossible. Of greater importance was the fact that considerable hemolysis was not detectable in deeply jaundiced serums, leading to large errors when the icteric indices were determined. They, therefore, sought a diluent which would precipitate proteins without affecting the color of the bilirubin. This would insure a clear filtrate free of hemoglobin, permitting accurate estimation of the icteric index. They added two parts of colorless redistilled acetone to one part of serum; the mixture was filtered and the color of the filtrate was compared with that of a freshly prepared standard solution of potassium dichromate, the serum being diluted further with pure ethyl alcohol until the colors matched. The authors suggested that specimens should be kept from light to prevent oxidation of the bilirubin to biliverdin, etc.

A simple and satisfactory modification of the acetone method of Ernst and Förster, used routinely in this laboratory, has been employed in this study.

*From the Laboratories of The Mount Sinai Hospital.
Received for publication, December 17, 1936.

METHOD

Freshly drawn clotted blood is centrifuged and 1 c.c. of serum is transferred to a graduated centrifuge tube. An equal quantity of colorless redistilled acetone is added. In the case of deeply jaundiced serums, three parts of acetone are added. The solution is mixed, allowed to stand for five minutes, and centrifuged. The supernatant fluid is pipetted off into a standard-sized test tube and compared with the standards in a comparometer box. For further dilution, if necessary, acetone is used. The same light source, preferably a strong daylight lamp, should be used for all determinations.

Standards are made up every few months and frequently checked against freshly prepared solutions. Ten standards of from one part to ten parts in 10,000 of potassium dichromate in distilled water are required. These are placed in the standard-sized test tubes, a drop of 0.1 N hydrochloric acid is added to each, and the tubes are then sealed.

Calculation: Icteric index = number of the standard used × dilution of original serum. For example, if a serum to which 3 parts of acetone were added matches standard No. 8 (eight parts of bichromate in 10,000), the icteric index is $4 \times 8 = 32$.

TABLE I

NUMBER OF OBSERVATIONS	MG. BILIRUBIN BY QUANTITATIVE VAN DEN BERGH (MG. PER CENT)	ICTERIC INDEX BY ACETONE METHOD			ICTERIC INDEX BY WATER METHOD		
		AVERAGE	LOWEST AND HIGHEST VALUES	MEAN VARIATION FROM AVERAGE	AVERAGE	LOWEST AND HIGHEST VALUES	MEAN VARIATION FROM AVERAGE
114	0.1- 0.5*	3.7	1-10	1.6	10.4	4-35*	4.0
34	0.6- 1.0	9.3	4-15	2.1	24.7	8-65	11.3
13	1.1- 1.5	13.8	10-20	1.9	39.1	23-75	9.7
16	1.6- 2.5	17.6	12-40	3.8	34.5	22-65	9.9
15	2.6- 5.0	24.7	15-36	3.6	66.7	28-105	19.1
30	5.1-16.0	39.6	19-70	10.0	120.6	40-185	30.4

*NOTE: Within the normal range (0.1-0.5 mg. per cent) of the quantitative van den Bergh reaction, the icteric index by the acetone method was greater than five in 20 cases, and greater than six in 12 cases; there were 38 values above ten (upper limit of normal) by the water method in this same range.

RESULTS

The table shows the variations and average values of the icteric index for various ranges of bilirubin concentration by the van den Bergh method. In each of the 222 analyses the quantitative van den Bergh reaction and the icteric index by both the saline* and the acetone methods were done. The table indicates that the acetone method gives values varying within a much narrower range than does the saline method for the same quantity of bilirubin by the van den Bergh test. The acetone method yields results averaging about one-third to one-half those obtained by the saline method. However, when serums contain over 5 mg. per cent of bilirubin, the icteric indices do not bear a constant relationship to the van den Bergh. Although by the water

*The saline method is similar to the acetone method except that precipitation of proteins is omitted and serums are compared directly with the standards. Dilution when necessary is carried out with normal saline (0.9 per cent) or distilled water.

method, the curve of averages more nearly approaches a straight line, the large individual variations (as indicated by the mean variation of over 30 units) obviate this advantage.

Values for icteric index above *five* obtained by the acetone method are regarded as abnormal although occasionally a normal serum will show an icteric index one or two units higher. When a slightly, but definitely, increased icteric index is unconfirmed by the van den Bergh test, the latter is regarded as probably being in error. This has been substantiated repeatedly by clinical observations. It has also been found that hemolysis does not interfere with the accuracy of the acetone method.

DISCUSSION

Because of its ease and accuracy, determination of the icteric index by the acetone method provides a convenient means for following the course of patients with jaundice of any type. In cases where differential diagnosis is required, the qualitative van den Bergh is essential. However, when the amount of serum bilirubin is small, determination of the acetone icteric index at frequent intervals provides more accurate information than the quantitative van den Bergh is capable of giving. An important practical advantage is the elimination of hemolysis as a source of error. Hemolysis accounts for some of the more flagrant inconsistencies seen between the quantitative van den Bergh and the icteric index by the saline method. When the amount of circulating bilirubin is high, the quantitative van den Bergh is more reliable than the icteric index.

Certain substances other than bilirubin have been thought to affect the icteric index, notably lipochromes, of which lutein and carotene are the most important. Meulengracht[3] pointed out that, while lutein occasionally colors the serum in certain animals, it has never been found sufficiently concentrated in human serum to influence the icteric index significantly. Nation and Myers,[4] investigating the question of carotenemia in 1934, found that in a series of 161 cases of which 75 were diabetics, the carotene concentration had a negligible effect (less than one unit) on the icteric index except in rare instances when there could be no question of the diagnosis. Dinitrophenol and atabrin may occasionally become sufficiently concentrated in the blood to cause an elevated icteric index without raising the van den Bergh value.

The icteric index is a valuable procedure if it is remembered that only the intensity of the yellow color in serum is measured.[5] In a given case changes in the color reflect the quantitative changes in the bilirubin. However, in comparing different cases, the color intensity may vary due to other factors, such as qualitative differences in the circulating bile pigments.

SUMMARY

The various methods for determining serum bilirubin have been considered. A modification of the acetone method of Ernst and Förster has been presented. The advantages of this method both technically and as an aid in following the progress of jaundice cases have been pointed out.

My thanks are due to Dr. Louis J. Soffer and Dr. Harry Sobotka for suggesting this investigation.

REFERENCES

1. Ernst, Z., and Förster, J.: Über die Bestimmung des Blutbilirubins, Klin. Wchnschr. **3**: 2386, 1924.
2. Van den Bergh, A. A. H.: Die Gallenfarbstoffe im Blute, Leipzig, 1918.
   Van den Bergh, A. A. H., and Grotepass, W.: An Improved Method for Determining Bilirubin in Blood, Brit. M. J. **1**: 1157, 1934.
3. Meulengracht, E.: Die klinische Bedeutung der Untersuchung auf Gallenfarbstoff im Blutserum, Deutsches Arch. f. Klin. Med. **132**: 285, 1920.
4. Nation, E., and Myers, V.: Carotenemia, Proc. Soc. Exper. Biol. & Med. **31**: 620, 1934.
5. Soffer, L. J.: Present Day Status of Liver Function Tests, Medicine **14**: 185, 1935.

A CRITICISM ON THE SUMNER METHOD FOR URINE SUGAR*

SAUL MALKIEL, M.A., BOSTON, MASS.

USE of the Sumner method for urine sugar indicated that normal urine constituents might interfere with the sugar analysis.

Solutions were prepared containing certain constituents of normal urine, glucose to known concentration added, and recovery attempted by the dinitrosalicylic acid reagent method of Sumner.[1] The reagent and method utilized were such as described[2] and later modified[1, 3] by Sumner.

Typical results as to the percentage of glucose recovered may be tabulated as shown in Table I.

TABLE I

SOLUTION INGREDIENTS	MG. ADDED	GLUCOSE RECOVERED	PER CENT RECOVERY
Urea	2.00	1.46	73.0
Urea	2.00	1.79	89.5
Urea	2.00	1.72	86.0
Uric acid	2.00	1.70	85.0
Uric acid	2.00	1.66	83.0
Uric acid	2.00	1.72	86.0
Creatinine	2.00	1.40	70.0
Creatinine	2.00	1.62	81.0
Creatinine	2.00	1.60	80.0
Urea, uric acid, creatinine	2.00	0.91	45.5
Urea, uric acid, creatinine	2.00	0.89	44.5
Urea, uric acid, creatinine	2.00	0.91	45.5
Urea, uric acid, creatinine, NaCl	2.00	0.89	44.5
Urea, uric acid, creatinine, NaCl, $CaCl_2$	2.00	0.83	41.5

To simulate normal urine the above constituents were added in these physiologic amounts: urea, 20 mg./ml.; uric acid, 0.5 mg./ml.; creatinine, 1 mg./ml.; sodium chloride, 10 mg./ml., and calcium chloride, 0.5 mg./ml.

The above analyses without the added glucose were also simultaneously carried out as controls and compared to a standard of blank reagent. Reduction to some degree was observed in these cases.

*From the Laboratory of Biochemistry, School of Medicine and the Department of Medical Sciences, Graduate School, Boston University.

Received for publication, December 8, 1936.

From these above results it may be concluded that the normal urine constituents interfere with the Sumner method for sugar.

Analyses were then conducted on normal urines as controls and the same urines to which a known concentration of glucose had been added. Results are given in Table II.

TABLE II

URINE	MG. AS GLUCOSE IN ORIGINAL	MG. GLUCOSE		MG. AS GLUCOSE RECOVERED	PER CENT RECOVERY
		ADDED	TO BE RECOVERED		
1	0.15	2.00	2.15	1.79	83.3
2	0.58	2.00	2.58	1.79	69.4
3	0.32	2.00	2.32	1.80	77.7
4	0.33	2.00	2.33	1.79	76.9
5	0.67	2.00	2.67	1.95	73.0
6	0.72	2.00	2.72	2.25	82.8
7	0.69	2.00	2.69	1.94	72.2
8	0.19	2.00	2.19	1.80	82.2
9	0.56	2.00	2.56	2.10	82.2
10	0.61	2.00	2.61	1.86	74.2
11	0.79	2.00	2.79	2.17	77.8
12	0.42	2.00	2.42	2.02	83.5

These results show that in actual urinalyses the constituent components of the urine hinder the actual sugar determination as performed by the Sumner method.

REFERENCES

1. Summer, J. B.: A More Specific Reagent for the Determination of Sugar in Urine, J. Biol. Chem. **65**: 393, 1925.
2. Summer, J. B.: Dinitrosalicylic Acid: A Reagent for the Estimation of Sugar in Normal and Diabetic Urine, J. Biol. Chem. **47**: 5, 1921.
3. Sumner, J. B.: The Estimation of Sugar in Diabetic Urine, Using Dinitrosalicylic Acid, J. Biol. Chem. **62**: 287, 1924-25.

CHEMICAL TESTS FOR THE DIAGNOSIS OF PREGNANCY*

ISADORE GERSH, M.D., AND JULIAN LEWIN, B.A., DENVER, COLO.

THE possibility of diagnosing pregnancy by chemical tests has interested the medical profession during the past few years. In an editorial of a recent issue of the *Journal of the American Medical Association*[1] the diagnosis of pregnancy by means of the bromine test for urinary histidine was discussed. It was pointed out that the usefulness of the test is not definitely known and further investigation was recommended to determine the value and significance of it. Another chemical test which also has aroused much interest was suggested by Visscher and Bowman.[2]

Voge[3] in 1930, using the bromine test for the detection of histidine in urine (Knoop reaction), found that his results closely paralleled those of the Aschheim-Zondek reaction. Burt-White[4] in 1930 obtained results similar to but less encouraging than those reported by Voge. G. H. Dodds[5] in 1930, after examining 380 samples of urine, concluded that the test is not specific for pregnancy. Armstrong and Walker[6] in 1932 showed that the Knoop reaction is quite specific for histidine and that only methyl-histidine gave a similar reaction. Kapeller-Adler[7] in 1933, with a quantitative method, determined the amount of urinary histidine excreted normally; in various diseases; and during pregnancy. Seidman[8] in 1935, with a modified Kapeller-Adler method, examined 199 samples of urine, and concluded that the excretion of histidine is an interesting biochemic phenomenon particularly characteristic of pregnancy, but that it is not specific enough to serve as a reliable test for this condition. Földes[9] in 1936, with his modification of the Kapeller-Adler test, reported 44 per cent positive results in a series of urinary specimens from 185 cases of pregnancy. Examination of another series of urinary samples from men and nonpregnant women showed 26.1 per cent positive reactions. He found that the incidence of histidine is greater as the specific gravity of the urine rises; also that histidine is more frequently present in concentrated specimens of urine from pregnant patients than in urinary specimens of similar concentration from men and nonpregnant women. Földes concluded that such a qualitative test is not of value for the diagnosis of pregnancy.

In 1934 Visscher and Bowman[2] developed a chemical test for pregnancy based on the probable oxidation of sex hormones in urine. They examined 317 specimens of urine from "established cases" with an accuracy of 93 per cent. Menken[10] with this test examined specimens of urine from nonpregnant women, known and questionable cases of pregnancy, and concluded that the

*From the Department of Clinical Pathology, University of Colorado School of Medicine.
Received for publication, December 19, 1936.

test is an aid to the practicing physician in the diagnosis of pregnancy. Dolff[11] used this test and obtained 96.08 per cent positive results in late pregnancies, 94.45 per cent in early pregnancies, and 81.82 per cent in ectopic pregnancies. He found that concentrated specimens of urine from men and from nonpregnant women, which contain reducing products of metabolism, often yield a positive reaction, and he suggested that more material be studied before conclusions are drawn regarding the value of the test.

We first made a comparative study of the tests as outlined by Voge, Kapeller-Adler, and Földes. From the results obtained, we concluded that the Voge test is the least efficient, giving the smallest number of correct positive results, and that the Kapeller-Adler method is less satisfactory than the procedure suggested by Földes.

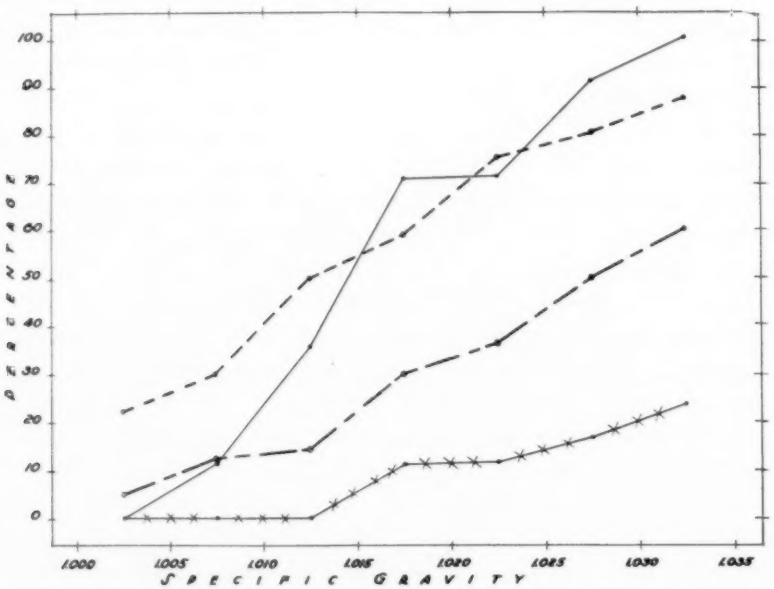


Chart 1.—Results of the Földes and Visscher-Bowman tests in terms of the percentage of positive results. ———— Földes test, and — — — — Visscher-Bowman test, with specimens of urine from cases of pregnancy. —×—×—×— Földes test, and —— — —— — Visscher-Bowman test, with specimens of urine from nonpregnant individuals.

The method of Földes was followed except that we omitted the use of potassium iodide starch solution as an indicator for the exact amount of bromine reagent to be used, because we found that this does not add materially to the sensitiveness of the test.

Following our preliminary work with the bromine tests a study was made to determine the comparative value of the Földes and Visscher-Bowman tests. The 748 samples of urine which we tested were received by the hospital laboratory for routine examination.

Table I shows the results of the Földes and Visscher-Bowman tests obtained with specimens of urine of varying specific gravity from cases of pregnancy of at least three months' duration, and Table II presents a similar study of urinary samples from men and nonpregnant women. Chart 1 records the results in terms of the percentage of correct and of false positive reactions.

TABLE I

RESULTS OBTAINED WITH URINARY SPECIMENS FROM CASES OF PREGNANCY

	SPECIFIC GRAVITY	LESS THAN 1.006	1.006 TO 1.010	1.010 TO 1.015	1.015 TO 1.020	1.020 TO 1.025	1.025 TO 1.030	1.030 TO 1.035	TOTAL
Földes test	Specimens of urine examined	42	59	56	41	31	11	7	247
	Number positive	0	7	20	29	22	10	7	95
Visscher-Bowman reaction	Specimens of urine examined	32	10	26	17	12	5	8	110
	Number positive	7	3	13	10	9	4	7	53

TABLE II

RESULTS OBTAINED WITH URINARY SPECIMENS FROM MEN AND NONPREGNANT WOMEN

	SPECIFIC GRAVITY	LESS THAN 1.006	1.006 TO 1.010	1.010 TO 1.015	1.015 TO 1.020	1.020 TO 1.025	1.025 TO 1.030	1.030 TO 1.035	TOTAL
Földes test	Specimens of urine examined	37	27	58	73	60	36	17	308
	Number positive	0	0	0	8	7	6	4	25
Visscher-Bowman reaction	Specimens of urine examined	21	8	14	20	11	4	5	83
	Number positive	1	1	2	6	4	2	3	19

DISCUSSION

From the results obtained it is evident that both tests yield a fairly high percentage of positive results with urinary specimens of high specific gravity from pregnant women, and that the proportion of positive results rises with increase in concentration of the urine. However, specimens of urine from nonpregnant individuals also yield an increasing percentage of positive results as the specific gravity rises. In our study, the Földes test yielded 13 per cent more false negatives and 14.9 per cent less false positives than did the Visscher-Bowman test. A positive Földes test with urine of specific gravity of 1.012 or less would be strongly indicative of pregnancy; on the other hand pregnancy would be unlikely when specimens of urine with a specific gravity of 1.032 or more give negative reactions (Chart 1). This would also apply to the Visscher-Bowman test but to a lesser degree.

It is evident that specimens of urine with a high specific gravity from both pregnant and nonpregnant individuals often contain a substance which yields a positive reaction with the Földes and Visscher-Bowman tests. Specimens of urine from pregnant women contain this substance more frequently, however, than do those of nonpregnant individuals.

CONCLUSIONS

1. Studies of the Voge, Kapeller-Adler, and Földes tests showed the last named test to be the most satisfactory.

2. Samples of urine with high specific gravities from normal individuals, and from pathologic states unrelated to pregnancy often yield positive Földes and Visscher-Bowman reactions.

3. The Földes test gives more false negative and less false positive reactions than does the Visscher-Bowman test.

4. Our results show that neither the Földes nor the Visscher-Bowman test is satisfactory for the diagnosis of pregnancy.

REFERENCES

1. Editorial: Urinary Histidine in the Diagnosis of Pregnancy, J. A. M. A. **106**: 2240, 1936.
2. Visscher, J. P., and Bowman, D. E.: Chemical Determination of Pregnancy, Proc. Soc. Exper. Biol. & Med. **31**: 460, 1934.
3. Voge, C. I. B.: A Simple Chemical Test for Pregnancy, Brit. M. J. **2**: 829, 1929; Proc. Roy. Soc. Med. **23**: 638, 1930.
4. Burt-White, H.: Discussion of paper by J. H. Hannan, entitled "The Detection of the Presence of the Hormone of the Anterior Pituitary Body in the Urine as an Aid to the Diagnosis of Pregnancy," Proc. Roy. Soc. Med. **23**: 639, 1930.
5. Dodds, G. H.: Value of the Bromine Test for Diagnosis of Pregnancy, Brit. M. J. **1**: 948, 1930.
6. Armstrong, A. R., and Walker, E.: The Bromine Reaction of Pregnancy Urine, Biochem. J. **26**: 143, 1932.
7. Kapeller-Adler, R.: Über eine neue Methode zur quantitativen Histidinbestimmung und über deren Anwendbarkeit zur Untersuchung von biologischen Flüssigkeiten, insbesondere von Gravidenharnen, Biochem. Ztschr. **264**: 131, 1933.
8. Seidman, T. R.: The Determination of Urinary Histidine as a Chemical Test for Pregnancy, Am. J. Obst. & Gynec. **29**: 451, 1935.
9. Földes, F.: Das Vorkommen des Histidinis im Menschlichen Urin, Biochem. Ztschr. **283**: 199, 1936.
10. Menken, J. G.: Chemical Pregnancy Reaction of Visscher & Bowman, Nederl. tijdschr. v. geneesk. **79**: 979, 1935. Abstr. Deutsche med. Wchnschr. **60**: 1837, 1934.
11. Dolff, C.: Bericht über einige Nachuntersuchungen der Chemischen Schwangerschaftsreaktion von Visscher & Bowman, Zentralbl. f. Gynäk. **59**: 2901, 1935.

DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

RED CELL SEDIMENTATION, Observations on, in Pulmonary Tuberculosis, Patterson, H. A. Am. Rev. Tubere. **34**: 164, 1936.

The results of the red cell sedimentation test in a series of 312 cases are reported. There were 1,698 sedimentation tests taken on 312 cases.

In 20 normal individuals the sedimentation index ranged from 1 to 7 mm., with an average of 3.5 mm.

The sedimentation rate as a whole was slower than that found at hospitals at a lower altitude. The altitude of this hospital is 6,231 feet.

There was a close relation between the sedimentation rate and the extent of pulmonary involvement.

The sedimentation rate was not proportional to the extent of pulmonary involvement in all cases reported in this paper.

Frequent sedimentation tests are advised after the patient has been discharged from the hospital.

BLOOD, pH of, in Pulmonary Tuberculosis, Wiese, E. R. Am. Rev. Tubere. **34**: 175, 1936.

Fifty tests were made on the blood of as many persons; of these, 45 specimens were blood of outspoken cases of pulmonary tuberculosis and 5 were from the blood of apparently normal, healthy persons. These were taken at a temperature of 23° C. and corrected to 38° C.

Only 9 cases (18 per cent) yielded the figure (7.40) usually considered the normal pH index of the blood.

There are no extreme variations from that of normal.

Such variations correspond in no wise to the physical condition of the patient.

The only logical conclusion seems to be that there is nothing in the hydrogen ion concentration of the blood of cases of pulmonary tuberculosis that is of diagnostic or prognostic value.

BLOOD SMEARS, Modified Wright's Method for Staining, Steil, W. N. Stain Techn. **2**: 99, 1936.

1. Place 4 or 5 drops of Wright's stain on slide with blood smear for one to two minutes.
2. Dilute the stain with an equal number of drops of neutral distilled water. Allow to remain for about three minutes.
3. Drain slide and flood it with neutral distilled water. Replace the water when the smear appears slightly pink. Drain off the water.
4. Place slide for a few minutes into a Coplin jar containing pure absolute methyl alcohol. Instead of placing the slide into a Coplin jar with the methyl alcohol, a few drops of the methyl alcohol may be placed on the smear.
5. Remove the absolute methyl alcohol with neutral distilled water. Replace with more of the distilled water, and allow to remain on the stained smear until a pinkish color appears. Remove portions of smear not desired. Examine slide.
6. Dehydrate with absolute ethyl alcohol.
7. Clear with clove oil.
8. Follow clearing with balsam.
9. Place cover slip on stained smear.

The use of neutral distilled water is very important. Ordinarily, freshly distilled water was sufficiently neutral for use. The pure absolute methyl alcohol removes at once the plasma stain, hence, a much better preparation may be obtained. The use of the absolute ethyl alcohol and clove oil insures a much more brilliantly stained smear.

DIABETES MELLITUS, Treatment of, With Insoluble Insulin Compounds, Gray, P. A. Endocrinol. 20: 461, 1936.

Insulin tannate is salt of insulin which is insoluble in the presence of sodium chloride at a pH acid to the isoelectric point. It is prepared by mixing in a hypodermic syringe equal volumes of U-100 strength insulin and a tannic acid solution. The resulting mixture contains 3 mg. of tannic acid per 100 c.c. It has about 20 to 25 per cent more hypoglycemic action per unit than commercial insulin.

Eighteen patients of various ages and with diabetes of varying degrees of severity have been treated with insulin tannate for periods varying from a few days to two weeks consecutively. Control of the diabetes has been possible when this compound is used alone or in combination with commercial insulin. Because of the relative insolubility of insulin tannate in human and animal tissues, delayed absorption and hence prolonged liberation of free insulin occurs. Increased effectiveness of insulin tannate over commercial insulin is shown by lower fasting blood sugar levels and smaller number of total units required per day when it is used. The hypoglycemic effect of insulin tannate has been compared with that of protamine insulinate and found to be of about the same order. The cheapness and availability of insulin tannate recommend it.

AORTA, I. Comparative Chemical and Histological Examinations of, for Calcium Content, Haythorn, S. R., Taylor, F. A., Crago, H. W., and Burrier, A. Z. Am. J. Path. 12: 283, 1936. **II. The Calcium Content of Arteriosclerotic Aortas, Haythorn, S. R., and Taylor, F. A.** Ibid. 12: 303, 1936.

Fifty-two aortas from patients between the ages of two months and seventy-eight years were analyzed chemically for metallic calcium and the results compared with the microchemical tests for calcium salts on the same aortas with the conclusion that:

1. None of the microchemical tests recommended for visible calcium gives more than a vague idea of the amount that can be recovered from the same aorta chemically. Von Kossa's silver method is not a specific stain for calcium, but it is the most satisfactory microscopic indicator of the comparative amounts of calcium deposited in sclerotic lesions.

2. Calcium deposits were brought out microchemically in only 1 of 20 aortas from cases under forty years of age, in 75 per cent of aortas from cases over forty years of age, and in 100 per cent of those over sixty years.

3. As age advances there is a consistent increase in the calcium content of aortas in excess of that of the bodily tissues generally.

4. Mild intimal lesions may occur without increased calcium by chemical analysis.

5. When the segments of all aortas from cases over forty years of age are averaged, the heaviest calcium deposits are found in abdominal portions.

6. In advanced aortic arteriosclerosis there is constantly found to be an overlapping of "type lesions," such as atheromatous cysts, diffuse medial calcification and plaque deposits. In this of all groups of lesions, the greatest inconsistencies between chemical and microscopic results occur.

7. In very advanced sclerotic lesions, characterized by heavy platelike calcium deposits, the chemical analyses yield the highest calcium values and the amounts are most nearly consistent when any 2 given sclerotic aortas are compared.

8. If the results obtained by chemical analysis are to be correlated with calcium which can be shown microscopically in sclerotic aortas, additional knowledge is required.

From the careful examination of 15 aortas selected according to the type of lesion present and in which detailed gross, microscopic and chemical calcium data were compared, the authors reached the following conclusions:

1. There is a consistent increase in the amount of calcium in the aorta as the years advance, which is independent of the "type lesion" present.

2. There is in addition an increase in the amount of calcium which depends upon the amount of arteriosclerosis present in the individual case and which varies with the kind and number of type lesions.

3. The type lesions of arteriosclerosis may be classified separately for study but each represents a step in a progressive disease and, as such, is rarely encountered singly in any given sclerotic aorta.

4. The increase in calcium appears to depend on two processes: that associated with elastin in the media, and that depending upon lipoidal degenerations in the contents of atheromatous cysts. The processes may operate singly but as a rule they go on simultaneously.

5. The highest calcium values were obtained from the calcium plates that appeared to be relatively stable end products. Such plates were most commonly found in the abdominal segments.

AGRANULOCYTOSIS, The Significance of the Monocytes in (Leucopenic Infectious Monocytosis), Rosenthal, N., and Abel, H. A. Am. J. Clin. Path. 6: 205, 1936.

A classification of agranulocytosis, based on hematologic findings, is suggested.

The authors have presented and discussed fourteen additional cases of leucopenic infectious monocytosis (agranulocytosis with marked monocytosis).

Leucopenic infectious monocytosis is apparently an infectious condition. A history of taking amidopyrine may be regarded as a predisposing factor in some of our cases; in others, atophan, bismuth, or arsenobenzol may have been responsible.

The principal symptoms are mild or severe infection with necrotic ulcerations on mucous membranes. The tongue and gums are usually involved. The course may be acute, recurrent, or chronic.

There is a leucopenia varying from 900 to 4,000 cells associated with a monocytosis at the onset of the disease. In three cases in our group, a leucopenia, neutropenia, and monocytosis persisted after the initial attack.

The prognosis is more favorable in agranulocytosis accompanied by monocytosis. In a series of 22 cases so far observed, 17 patients have recovered (77 per cent).

SPIROCHETAL JAUNDICE, Silver Impregnation of L. Icterohaemorrhagiae, Murray, R. E., and Fielding, J. Med. J. Australia 1: 610, 1936.

1. Cut strips of tissue 1.5 mm. thick.
2. Fix one hour at 50° C. or eighteen hours at room temperature in (a) F.A.A. (formol, 5; acetic acid, 3; absolute alcohol, 46; distilled water, 46); or (b) F.A.S. (formol, 5; acetic acid, 3; saline solution, 92).
3. 70 per cent alcohol, thirty minutes at 50° C.
4. 1 per cent silver nitrate, thirty minutes at 50° C.
5. Wash in five to six changes of distilled water.
6. Develop sixty minutes at 50° C. in (a) 1 per cent pyrogallic acid in 5 per cent formalin, (b) 1 per cent pyrogallic acid, or (c) 1 per cent quinol.
7. Wash in five to six changes of distilled water.
8. 50 per cent alcohol, fifteen minutes at 50° C.
9. 70 per cent alcohol, fifteen minutes at 50° C.
10. 90 per cent alcohol, fifteen minutes at 50° C.
11. Absolute alcohol, fifteen minutes at 50° C.
12. Repeat absolute alcohol, fifteen minutes at 50° C.
13. Xylol, fifteen minutes at 50° C.

14. 46° to 48° C. melting point paraffin, fifteen minutes.
15. 50° to 52° C. melting point paraffin, fifteen minutes.
16. Embed in a suitable paraffin.
17. Cool block in water or on ice.
18. Trim paraffin block.
19. Mount on wood fiber block.
20. Cut sections 4 to 6 microns in thickness.
21. Float sections on water (albuminized slide).
22. Flatten by gently heating in Bunsen flame.
23. Drain water, blot and dry in oven.
24. Dissolve paraffin with xylol.
25. Mount in balsam.
26. Examine under oil immersion lens.

Points To Be Observed In The Silver Impregnation Technic For Leptospirae:

1. Do not fix in bulk, but cut into strips about 1.5 to 2.0 mm. thick.
2. Do not mix formalin with tap water.
3. Do not fix in alkaline fixatives.
4. Do not wash in tap water at any stage.
5. Do not leave the tissues in the fixative, but store in 70 per cent alcohol.
6. Do not forget to have all required solutions at 50° C. on transfer of tissues.

TISSUE: Method of Temporarily Preserving Fresh Frozen Sections Stained With Polychrome Methylene Blue, Kernohan, J. W. Am. J. Clin. Path. 6: 185, 1936.

The method employed is, as soon as the diagnosis is arrived at and before the mounting fluid (glucose or water) has evaporated, to paint around the edge of the coverslip with a clear, quick-drying lacquer (clear "Duco"). This serves to prevent evaporation of the mounting fluid and to seal it. The blue will of course ultimately (after a few days or weeks) go into solution in the mounting medium; but fixed sections have by that time been prepared and examined.

The use of a clear, quick-drying lacquer is not limited to fresh frozen sections, as all microscopic preparations mounted in balsam, or some modification of this mounting medium, can be sealed with the preparation. It is also useful in preserving fat stains by surrounding the cover glass, thus preserving the section indefinitely.

RENAL FUNCTION, Evaluation of Measures of, in Persons With Arteriosclerotic Bright's Disease, Elliot, A. H., and Nuzum, F. R. Arch. Int. Med. 57: 1152, 1936.

In 111 cases of arteriosclerotic Bright's disease renal function was measured by the phenolsulphonphthalein test and by the dilution and concentration test of Volhard. In addition, the urea clearance was determined in 33 patients, creatinine excretion (Major's test) in 34 and the urea nitrogen level of the blood in 67.

Impairment of diluting ability was present in one-fourth of the patients with essential hypertension. The creatinine excretion was low in one-third. Urea clearance, concentrating ability and phenolsulphonphthalein output were each low in one-half of the patients on whom these measurements were made.

Correlation between the results of the tests was vague and little better than could be expected as a chance occurrence. In the individual instance, it was impossible in this study to predict the outcome of any one test on the basis of the result given by another. A possible exception to this was that an increased amount of urea nitrogen in the blood was usually accompanied by a decreased output of phenolsulphonphthalein.

The effect of weakness of the left ventricle on the results of the tests was studied in 21 instances. The phenolsulphonphthalein output was not apparently decreased; the urea clearance was. By the Volhard technique, a lag in the excretion of water and an impairment of concentrating ability were demonstrated in these patients.

The significance of nocturia in patients with weakness of the left side of the heart, traceable to a delay in the diuretic response to fluids consumed in the latter part of the day, is discussed. When shown without obvious cause by such patients, nocturnal polyuria is primarily of cardiac origin, and its presence may be of value in the diagnosis of impending failure of the left ventricle.

It is difficult to interpret the results of tests of renal function in persons with arteriosclerotic renal disease in terms of the actual amount of healthy renal parenchyma. The value of the available tests as an aid to prognosis is disappointing.

PLATELET COUNT, The Differential, Olef, I. Arch. Int. Med. **57**: 1163, 1936.

The preserving fluid employed in this investigation for both total and differential platelet counting has the following composition:

	gm. or c.c.
Sodium metaphosphate	1.0
Sodium chloride	0.5
Dextrose	0.1
Distilled water	100.0

Under optimal conditions the platelets in this solution, when observed with the oil immersion lens, appear as free-floating, isolated, clear, highly refractile bodies with numerous spinelike processes projecting from the periphery. Most of the platelets are fairly round or ovoid; irregularly shaped thrombocytes, especially elongated forms considered by Aynaud to be the common physiologic type, are seen rather infrequently in these preparations. If it is desirable to stain the platelets the following solution may be used:

	gm. or c.c.
Sodium metaphosphate	1.00
Sodium chloride	0.40
Dextrose	0.10
Sodium bicarbonate	0.10
Brilliant cresyl blue	0.15
Distilled water	100.0

The palmar surface of the fingertip is punctured with an automatic lancet after thorough cleansing of the parts with soap and water and subsequent drying with alcohol and ether. The first drop or two of blood is discarded. A drop of the diluting fluid is then placed over the puncture wound before the blood reaches the surface of the skin, and the hand is quickly turned over so that the palmar surface is directed downward. After a sufficiently large drop of blood has escaped into the drop of diluent, the mixture is applied to the surface of a small quantity (3 or 4 drops) of diluting fluid contained in a paraffin cup. The mixture is gently stirred and then transferred by means of a paraffin-coated applicator to a glass slide; usually three preparations can be obtained, as the quantity of fluid in the cup yields 3 large drops. A cover slip is placed over each drop, and after the preparations have been allowed to stand for from ten to fifteen minutes a relative thrombocyte-erythrocyte count is made, the oil immersion lens being used. Sealing the edges of the preparations with liquid petrolatum will prevent air currents in them. An erythrocyte count is then done in the usual manner, and the absolute number of platelets per cubic millimeter is determined. With this method the average number of platelets per cubic millimeter in normal adults is about 500,000.

The normal platelets can be differentiated into four groups according to size: Group 1, consisting of platelets 1.8 microns in diameter; Group 2, consisting of platelets 2.5 microns in diameter; Group 3, consisting of platelets 3.6 microns or more in diameter, and Group 4, consisting of irregular-shaped platelets. In normal persons 18.6 per cent of all the circulating platelets belong to Group 1, 63.3 per cent to Group 2, 17.4 per cent to Group 3 and 0.7 per cent to Group 4.

In conditions associated with thrombocytosis and at times in those associated with thrombopenia, the deviation from the normal in the differential platelet formula is usually

due to an absolute or relative increase in the number of small platelets; i.e., those belonging to Group 1. The presence of increased numbers of the larger types of platelets is often associated with intense regenerative activity, abnormal function or hypoplasia of the megakaryocytes in the bone marrow.

Normally functioning platelets are usually normal morphologically. Functionally, the smaller platelets are much more active than the larger types. The small juvenile platelets possess high agglutinating powers, and their presence in large numbers constitutes a significant factor in the causation of spontaneous thrombosis in conditions associated with thrombocytosis.

CIRCULATION TIME, The Use of Calcium Gluconate as a Test for, Goldberg, S. J. Am. J. M. Sc. **192:** 36, 1936.

The arm-to-tongue circulation time, a relative index of the rate of blood flow through the lungs, is a useful test of the functional efficiency of the circulation. The velocity with which the blood flows through the lungs is a composite result of the oxygen demands of the body and the capacity of the heart and blood vessels to propel the blood to meet such demands.

A simple method is described which lends itself to general use, and the results in 156 normal and pathologic cases are reported. The calcium gluconate method gives normal readings varying from 10 to 16 seconds, with an average arm-to-tongue circulation time of 12.5 seconds.

Hyperthyroidism increases the velocity of the blood flow, and cardiac failure markedly slows it. In myxedema and hypothyroidism there is a slowing of the circulation in proportion to the fall in metabolism. The test is helpful in the differentiation of edema of cardiac or renal origin, and may be of assistance in distinguishing between cardiac and bronchial asthma. The calcium gluconate test is approximately accurate, and easy to perform. No untoward effects have been observed, nor has there been any thrombosis or tissue damage in a single instance

The method follows: The patient reclines, with the arm at the level of the right auricle. After entering the vein, the tourniquet is withdrawn, and the circulation is permitted to return to normal. A stopwatch is held in the left hand, and 3 to 5 c.c. of the 10 per cent solution is injected as rapidly as possible with the other, through an 18 gauge needle. When the larger amount is used, a characteristic response is invariably elicited. The time required for completing the injection is subtracted from the total circulation time, although if a needle of large bore be used, this rarely occupies more than one-half second. There has been recently made available a 20 per cent solution of calcium gluconate which permits the use of smaller volumes, and minimizes the factor of injection time. Recent tests have been carried out with 2.5 c.c. of this solution with improved results. There is no pain, thrombosis or slough should the injection be improperly made. The patient announces the onset of the hot sensation in the pharynx by crying out "hot," or some other such signal. The sensation is sudden in onset, and wells up rapidly into the throat very much like a "gust of steam," to use the expression of one patient. It is next felt in the face, and then successively in the anterior chest, perineum, the hands, and finally the feet. These effects depend upon the arrival of the solution in the respective peripheral arterial beds. The onset of the sensation in the throat and tongue is most intense, as the substance is here present in greatest concentration.

After the sensation has entirely subsided, usually within one or two minutes, the reading may be repeated, without removing the needle from the vein. The second reading usually checks closely with the first, indicating that the first injection of the material has no great effect on the circulation itself. No discomfort is experienced by the patient as a result of the rapid injection, even in those with advanced heart failure.

CHLORIDES, Urinary, Determination of, Fantus, B. J. A. M. A. **107**: 14, 1936.

The following test is described in a paper on the postoperative use of fluid (fluid postoperatively).

Place 10 drops of urine in a test tube. Add 1 drop of a 1 to 5 potassium chromate solution. The fluid will now assume a somewhat distinctly yellow color. Add drop by drop, with the same dropper or one of the same caliber, a 2.9 per cent silver nitrate solution until a permanent and distinct color change to red brown occurs (resulting from the formation of silver chromate). The number of drops required to produce the change of color expresses in grams the content of chloride per liter of urine.

HYPERCALCEMIA, Induced, Its Possible Therapeutic Relation to Thrombocytopenic Purpura, Lowenburg, H. Sr., and Ginsburg, T. M. J. A. M. A. **106**: 21, 1779.

A second case of acute hypercalcemia produced by intentional overdosage with parathyroid extract occurred in a boy with thrombocytopenic purpura.

Toxic symptoms occurring in man are similar to those reported by many workers as occurring in animals with experimental hypercalcemia.

The earliest symptom is vomiting. This is followed shortly by weakness, apathy, and lethargy.

Both patients, once hypercalcemia was established, presented definite objective changes in the blood (bleeding time, clotting time, clot retraction, platelet count) as well as clinical cure.

A cause-and-effect relationship between the hypercalcemia and the apparent cures is suggested, although there appear to be no sound theoretical grounds for such a conclusion.

Calcium gluconate was used to protect the bones from the withdrawal of calcium from them into the blood.

HODGKIN'S DISEASE, The "Gordon Test" for, Goldstein, J. D. Am. J. M. Sc. **191**: 775, 1936.

Biopsy material from 7 to 9 cases of Hodgkin's disease produced an encephalitic syndrome (a positive "Gordon test") when inoculated intracerebrally into rabbits.

Twenty control lymph nodes, including 5 tuberculous nodes and 2 nodes from infectious mononucleosis, were negative.

There were no false positive tests. Two of 9 nodes from established cases of Hodgkin's disease failed to give a positive test.

TYPHOID, Production and Persistence of H & O Agglutinins and Complement-Fixing Bodies in Persons Inoculated With Typhoid Endotoxoid Vaccine, Grasset, E., and Lewin, W. Brit. J. Exper. Path. **17**: 179, 1936.

The sera of individuals inoculated with typhoid endotoxoid vaccine produced by the South African Institute for Medical Research showed the presence of H and O agglutinins, in the large majority of cases in high titer. A concentrated antigen produced a higher O agglutinin response than this regular product.

The titer for H agglutinins shortly after inoculation is much higher than for O, but the titer of the latter remains at a more constant level, the H titer falling rapidly.

At some stage during inoculation complement-fixing bodies were present in approximately 32 p.c. of individuals inoculated with endotoxoid vaccine of a concentration as issued for human use. A more concentrated antigen, in a series of 7 individuals, produced a positive complement fixation reaction in all cases.

Of sera from 33 cases examined seven months after the last injection, 4, or 12 p.c., gave a positive complement fixation reaction.

An O agglutination test performed in a serum dilution of 1 in 200 will not aid the diagnosis in the case of a person with a febrile illness who has been recently inoculated with typhoid endotoxoid vaccine. Inoculation of a population with such a vaccine will limit the use of H and O agglutination tests and the typhoid complement fixation test for the detection of the carrier state.

SYPHILIS, Congenital, Quantitative Wassermann Tests in the Diagnosis of, Faber, H. K., and Black, W. C. Am. J. Dis. Child. **51**: 1259, 1936.

Additional proof is offered that syphilitic reagin in the blood of the newborn infant is diagnostic not of syphilis in the infant but of syphilis in the mother. For this phenomenon the authors propose the name "Fildes' law" from its discoverer.

In the absence of definite evidence of syphilitic infection, a positive reaction to the ordinary qualitative test is not sufficient justification for subjecting the infant to the risks of arsphenamine therapy.

For infants with an initially positive reaction to tests made by the routine method, the quantitative method, in which the amount of reagin present is measured, is a valuable means of distinguishing between those who have and those who have not syphilitic infection. In the latter the amount of reagin is usually, but not always, small and, in any case, progressively declines to zero.

The decline occurs early and, in the authors' experience, can be detected by the end of the first week or sooner, whereas a Wassermann test made by the ordinary method may fail to show any evidence of weakening till much later. In one case, the titer was found to be declining on the seventh day but did not reach zero until the ninety-fourth day, and the reaction to the routine test remained "strongly" positive (+++) until the seventy-third day.

ACIDOSIS, Diabetic, Treatment of Severe, Hartman, A. F. Arch. Int. Med. **56**: 414, 1935.

As a result of a critical study on eighty-six patients with diabetic acidosis treated at the St. Louis Children's Hospital during the past twelve years the author has come to the conclusion that the most effective method of treatment demands the use of alkali (sodium bicarbonate or racemic sodium lactate), together with insulin, Ringer's solution and solution of dextrose. When sodium lactate is used instead of sodium bicarbonate, the dextrose solution may be omitted. The method of treatment of severe diabetic acidosis which is recommended at the present time includes:

1. Immediate parenteral administration (one-half intravenously, the remainder subcutaneously and intraperitoneally) of 60 c.c. of a sixth molar solution of racemic sodium lactate per kilogram of body weight.
2. Immediate administration of 2 units of insulin per kilogram of body weight.
3. Administration of 40 c.c. of Ringer's solution per kilogram of body weight as soon after the administration of sodium lactate as possible.
4. Repeated administration of insulin six hours later in a dose of one-half unit per kilogram of body weight.
5. Transfusion of citrated whole blood or plasma (20 c.c. per kilogram of body weight) if edema due to reduced plasma protein develops.